CIRCADIAN RHYTHMS IN PLANTS, INSECTS AND MAMMALS EXPOSED TO ELF MAGNETIC AND/OR ELECTRIC FIELDS AND CURRENTS

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Circadian Rhythms in Plants, Insects and Mammals Exposed to ELF Magnetic and/or Electric Fields and Currents

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ABSTRACT

Proceeding on the basis of knowledge that circadian rhythms are a predictable source of biological variability with characteristics that can change in response to potentially harmful agents, studies were performed on plants, insects and mammals in the presence and absence of ELF fields and currents. Specifically, circadian rhythms were examined in leaf movements of Albizzia julibrissin (silk tree), in susceptibility of Tribolium confusum (flour beetle) to an insecticide, in body temperature and drug resistance of Mus musculus (mouse). In the latter animal body weight, food consumption, the estrus cycle and survival were also investigated.

Field conditions ranged from 45 to 75 Hz, 0.4 to 2 gauss and 1 to 180 v/m. Duration of field exposure varied from a few days to several months. Such exposure was consistent with the demonstration of statistically significant circadian rhythms. Characteristics of these rhythms, as compared to other endpoints examined, are regarded as sensitive indices of any environmental effects. These studies did not lead to the detection of any clearly harmful effects.

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I) Introduction.

With one notable exception (1) studies dealing with effects of magnetic and electric fields upon organisms (2-6) have not considered biological rhythmicity. Circadian rhythms are a prominent characteristic in all life forms investigated (7). Essentially all variables examined, whether classified as physiological, biochemical or pharmacological, exhibit about-24-hour periodicity. The existence of such predictable variability must be considered in the design of experiments. For example, the time at which a drug is administered to an animal may be as important a consideration as its dose. Furthermore, it may not be sufficient to simply control the time at which an observation is made when one is investigating an organism's response to a possibly harmful agent. The agent may alter the characteristics (e.g., the timing) of a rhythm in that variable, as has been demonstrated in the case of mice treated with a carcinostatic drug (8). Characteristics of these rhythms--their overall average value, their amplitude and timing--may in themselves be sensitive indices of response. It is against this background that we studied effects of ELF fields and currents on a plant (Albizzia julibrissin), an insect (Tribolium confusum) and a mammal (Mus musculus).

II) Investigations on a plant: Albizzia julibrissin.

The "silk-tree", Albizzia julibrissin, exhibits a dramatic circadian rhythm in leaf movement whether in a daily light:dark cycle or in continuous light. Such movements continue even when the pinnules (leaflets) of the compound leaf are excised and maintained "in vitro" in an appropriate medium. These properties make this plant an especially suitable subject for a study of possible effects of ELF fields on circadian rhythms.

Methods and Results

Twelve experiments were performed, on excised pinnules "in vitro" and on entire plants "in vivo". For in vitro studies of magnetic field effects 4 Helmholz coil systems were used. Each system consisted of 3 coils, 40 cm diameter, "stacked" 20 cm apart in an aluminum frame. The upper and lower coils contained 50 turns and the middle coil 25 turns of #14 magnet wire. The magnetic field in the space occupied by the pinnules had a variation of less than 5% at 1 gauss field strength. For in vivo experiments, each coil system consisted of 2 coils approximately 40 cm apart, with a diameter of 110 cm. Coils had 25 turns of #14 magnet wire and could be driven either in parallel or in series. Exposure to electric fields and currents was by means of metal plates. In experiment 10 (in vivo) these plates extended into the soil to become electrodes for current. Resistivity was monitored and controlled to some extent by adjusting the moisture content of the soil.

The experiments and their results are summarized below, in tabular form (Tables 1 and 2) and in Figures 1-11:

Experiment 1: Forty silk tree pinnules were excised from two plants which had been standardized for four days on a lighting regimen of 12 hours light, from 06^{00} to 18^{00}, alternating with 12 hours darkness--L (06-18). Fourteen of these pinnules (Group A) were placed in individual Petri dishes containing Hoaglund solution and placed in a 60 Hz magnetic field of approximately 1 gauss. Twenty-six pinnules (Group B) were placed in a similar in vitro condition without the magnetic field, to serve as controls. In an LL condition pinnule angles (the angle between two opposite pinnules) were measured about every 1-1½ hours for 36 hours. These angles could range from 0° when the pinnules were completely closed to 180° when fully open. Data on pinnule angle as a function of time were fitted with a 24-hour cosine curve by the method of least squares (9),

to yield parameter estimates for the circadian rhythm in pinnule movement: Mesor (rhythm-average angle), Amplitude (half the extreme variation in angle) and Acrophase (timing of greatest angle). Figure 1 presents the results obtained from groups A and B in "cosinor" form. In this polar presentation the outermost circular scale is in clock hours and the inner scale is in degrees in relation to midnight (360° = 24 hours). The direction, in relation to these scales, of lines originating at the center of the circle (vectors) indicate the acrophase of the rhythm. For example, the high point of rhythms in both groups occurred about noon. The length of each vector represents the rhythm's amplitude (in this case as a percentage of the mesor). The ellipse at the tip of each vector indicates its 95% confidence region. If this region does not include the pole (center of circle)—i.e., zero amplitude—we conclude that a statistically-significant rhythm has been demonstrated. These parameter estimates, along with those of the mesor (level) are also presented numerically in a table at the bottom and in bar graphs at the right of the Figure. The results suggest that group A (field-exposed) exhibited an increased mesor, lower amplitude (as per cent of mesor) and a slight phase-shift of the circadian rhythm in pinnule movement, as compared to controls.

Experiment 2: This study was designed to replicate Experiment 1. Twenty four pinnules were excised from two plants and their angles measured in LL about every 1-1½ hours for 80 hours--twelve pinnules each in the experimental and control groups. Cosinor analysis showed similar values for the mesor and amplitude of each group but an advanced phase for the experimental pinnules, in contrast with the delayed phase for the experimental group in the first study (Table 2).

Experiment 3: Pinnules were excised from two plants which had been in an LL condition for 7 days. Twelve pinnules each were assigned to the experimental and control groups (A and B respectively) and measured about every 1-1½ hours in an LL condition for 107 hours in petri dishes similar to Experiment 1. Cosinor results (Figure 2) show the expected phase-drift in an LL condition for both groups in comparison with results shown in Figure 1. An increase in mesor and a decrease in amplitude of the circadian rhythm were detected for those pinnules in the magnetic field of approximately 1 gauss.

Experiment 4: Twenty four pinnules from one plant standardized in L (07-19) for 3 days were excised and placed individually in petri dishes, this time on a filter paper block soaked with Hoaglund solution. Twelve pinnules were placed in the magnetic coil structure and twelve served as controls. For 49 hours both groups were measured in this pre-exposure condition to establish control parameter estimates. Identical mesors, amplitudes and phases were found through cosinor analysis of these control data. Subsequently, the magnetic field of 1 gauss was turned on and both groups measured for another 29 hours each hour and a half. Cosinor analysis of the exposure span again indicated a similarity of circadian rhythms for the control and experimental pinnules (Table 2).

Experiment 5: (Combined magnetic and electric fields): Pinnules were excised from two plants standardized in L(07-19) for 10 and 24 days and placed in a slightly altered feeding system. Filter blocks soaked with Hoaglund solution were used as support at each end of a stem containing four pinnule pairs. Six of these "groups" (24 pinnules) were used in the experimental condition (Group B) and five (20 pinnules) served as controls (Group A). Pre-exposure

study was for 43 hours in L(07-19) with pinnule-angle measures each 1-1½ hours in the light and each 3 hours in the dark span. Cosinor analysis shows similar parameter estimates for the circadian rhythms of both groups (Figure 3). The exposure condition for the experimental group consisted of a 60 Hz magnetic field which began at 1.0 gauss but was accidently altered to .5 gauss and an electric field of 100 V/m from two copper plates set in each petri dish parallel to the stem of the excised leaf, one on either side. The control group also had the two copper plates in the dishes but with no current. Angles were measured for 72 hours in L(07-19). Cosinor analysis (Figure 4) showed an increased mesor, a drastically reduced amplitude but with no change in acrophase during exposure to these fields. It is also important to note that the physical condition of the experimental pinnules seemed to deteriorate much more rapidly than the controls, showing chlorophyll loss, drooping and curling of the tips, but the accidental nature of this finding is definitely not ruled out.

Experiment 6: This experiment was designed as a repeat of Experiment 5. Pinnules were excised from the same two plants used in Experiment 5, now standardized for 15 and 29 days in L(07-19). The filter block feeding system was again used to support the pinnule stem in vitro (see Figure 5). 24 pinnules were used as experimentals and 20 as controls. Pre-exposure lasted 44 hours in L(07-19) with angles measured every 1-1½ hours during light and 3-hourly in darkness. Cosinor analysis showed identical results between the two groups during this control span. The exposure to magnetic and electric fields was identical to that in Experiment 5 except that the 1 gauss magnetic field lasted for 64 hours. Cosinor analysis again showed a greatly increased level and decreased amplitude (Table 2). Again the experimental pinnules deteriorated much faster than the controls, as shown by drooping, curling edges and loss of chlorophyll.

Experiment 7: This experiment was designed to study the effect of electric fields with different intensities (0, 1, 10, 100, 180 V/m) upon the circadian rhythm in pinnule movement. 60 pinnules were excised from two plants, standardized for 8 days in L(07-19). Each of five "groups" consisted of 12 pinnules (3 stems with 4 pinnules each) supported by the filter-block feeding system in a row between two stainless steel plates, for a total of 5 rows (Figure 6). Pre-exposure lasted for 54 hours with angles measured about every 1-1½ hours during the light and about every 3 hours in the dark span. Cosinor analysis established baseline circadian parameters for each of the five groups, the timing being nearly identical and average mesors only slightly spread apart (Table 2). Exposure conditions consisted of an electric field of 0 (control), 1, 10, 100 or 180 volts/meter in the open space between the metal plates. Angles were measured at 1-1½ to 3 hour intervals for 37 hours. Cosinor analyses (Figure 7 and Table 2) showed an advanced acrophase for the control group (0 V/m), in comparison to pre-exposure results, while those groups with electric fields of different intensities remained closely synchronized with their pre-exposure estimates. All groups, including the control, showed an increase in mesor and decrease in amplitude of the circadian rhythm, with the experimental group in the 100 V/m field retaining an amplitude somewhat larger than all other groups (Figure 7D).

Experiment 8: This experiment was designed to investigate the effect of a magnetic field on the pinnule angle rhythm on a plant in vivo. A plant was standardized for 3 days in L(07-19), then placed inside the magnetic coil apparatus for 116 hours of pre-exposure measurements. 8 pinnules each from leaves 1 and 2 were measured. Cosinor analysis showed nearly identical parameter estimates for the two leaves (Table 2). The exposure condition consisted of a

magnetic field of 1 gauss surrounding the entire plant. Angles were measured every 1 to 3 hours for 116 hours in L(07-19). Cosinor analysis showed the persistence of a circadian rhythm at a high level and amplitude with a slightly delayed phase. The plant set-up was subsequently moved to another room, undergoing a 3-hour phase-shift. After 13 days, the magnetic field was again turned on and the angles monitored for the next 19 days. A "serial section analysis" of the entire experiment is shown in Figure 8.

The scale across the bottom of this figure is graduated in days of the study. Ordinate scales for each of the 5 rows of the figure are indicated on the right side. The top row presents raw data (pinnule angles) as a function of time. The second (narrow) row gives P-values used in judging whether a 24-hour cosine curve is a good approximation to the data--P-values $\leq .05$ are considered favorable to the model. Parameter estimates from this fitting process, carried out in the nature of a "moving-average" throughout the entire series of data, are plotted in rows 3 and 4. Mesor (level) estimates are plotted on the bottom curve in row 3 while amplitude estimates are presented as the separation between the bottom and top curves. Acrophase values are plotted in row 4. Dots above and below these 3 curves are dispersion indicators. Finally, in the fifth (narrow) row, the number of data available for each of the serial estimations is plotted. Vertical dashed lines indicate the time of events. Noteworthy is the absence of any marked change in parameters when the field was imposed (at event lines 1 and 4).

Experiment 9: This experiment was designed to investigate the effect of an electric current running directly through the stem of the excised leaf. Using the same board as in Experiment 7, 15 filter block units were set up, each unit containing 4 pinnules (for a total of 60 pinnule pairs) from a plant standardized

for 10 days in L(10-22). Two rows containing 6 filter-block units were connected by thin silver wires between the blocks and served as controls (no current). Three rows with 9 filter-block units were connected by thin silver wires through which a current of 300 pA was passed. Pre-exposure conditions lasted 40 hours in L(10-22) with measurements every 1 to 3 hours. Cosinor analysis showed similar circadian parameter estimates for both groups. With the electric current on, the exposure condition lasted 46 hours. Cosinor analysis showed a decreased mesor for circadian rhythms of the experimental group, while both groups showed an acrophase later than that observed before exposure (Table 2).

Experiment 10: This experiment was designed to study the combined effect of a magnetic field, an electric field and an electric current in the soil on pinnule rhythms of plants in vivo. Four plants synchronized for one week in L(07-19) were transplanted individually from pots to large boxes filled with soil. Stainless steel plates on opposite sides of each box extended from the inside bottom of the box up over the height of the plant. This system was surrounded by wire coils which were used to create the magnetic field. There were two plants each in similar set-ups for the experimental and control groups. Pre-exposure conditions lasted 172 hours in L(07-19) with measurements every 1 to 3 hours. Cosinor results show similar parameter estimates for the two groups prior to exposure. The experimental condition consisted of a magnetic field of 1 gauss, electric field of 100 v/m and electric current of 300 pA for the two experimental plants and lasted 305 hours in L(07-19). Circadian cosinor results were similar to pre-exposure estimates for both groups (Table 2).

Experiment 11: An in vitro and an in vivo study of pinnule responses to a 60 Hz, 1 gauss field were performed concurrently. Sixteen pinnule-pairs were

excised and placed in petri dishes. Pinnule angles were measured on these 16 pairs in vitro and on 4 pairs on 2 intact plants during a 24-hour span to establish a pre-field baseline. Following this, 8 of the in vitro pinnule-pairs and 1 of the intact plants were exposed to the magnetic field. Measurement of pinnule angles continued for 3.5 days. Results from the in vitro and in vivo measurements are plotted in Figures 9 and 10, respectively. Field-exposed and control pinnules behaved identically in vitro, both before and after the field was turned on. The 2 intact plants yielded slightly different results in terms of the extent of circadian variations in pinnule angle before as well as after onset of the magnetic field.

Experiment 12: This was an in vitro study of effects from combined electric and magnetic fields at 60 Hz. Three sets of pinnule pairs were involved. Measurement of pinnule angle was first performed on all pinnule pairs during a 24-hour span to establish a baseline. One set (of 16 pinnule pairs) was then exposed to a 0.4 gauss magnetic field and a 100 V/m electric field. A second set (8 pinnule pairs) was subjected to a 1 gauss magnetic field plus a 100 V/m electric field. The third set (8 pinnule pairs) continued without any applied field, as a control. Pinnule angle measurements were continued for 11 days after fields were turned on. Magnetic fields were inadvertently interrupted for about a day on the third day of field exposure. Results of pinnule angle measurment during the first 7 days of the study are presented in Figure 11. A progressive difference among the 3 sets of pinnule-pairs is evident beginning about the third day of exposure to fields. There is a gradual increase in the 24-hour average pinnule angle and a gradual decrease in the amplitude of the rhythmic movement, indicating that pinnules are not moving as much from the completely open position. This change is particularly marked in the case of pinnules exposed to the 0.4 gauss field. Pinnules exposed to the 0.4 gauss

field also had a shorter survival time, as determined by the time at which pinnules drop from their stems. These results indicate that a 0.4 gauss field, combined with a 100 V/m electric field may be more harmful to the pinnules than is the combination of a 1 gauss field and 100 V/m electric field.

In summary of these plant studies we did not establish, in the case of the intact plant (in vivo experiments), any marked effect of a 60 Hz, 1 gauss magnetic field--Experiment 8--nor of a combined 60 Hz magnetic field (1 gauss), electric field (100 V/m) and ground current (300 pA)--Experiment 10--on the circadian rhythm in pinnule movement.

The in vitro investigations, as a whole, were inconclusive. Among those in which pinnules were exposed to a magnetic field only (Experiments 1-4), two studies (1 and 3) indicated a marked increase in the rhythm's mesor (average pinnule angle). In Experiment 3 the amplitude of the rhythm was also depressed considerably during field-exposure. On the other hand, there was no statistically significant difference in mesor between control and experimental pinnules in Experiments 2 and 4, nor was the amplitude decreased. A lack of agreement among experiments in which pinnules were exposed to the same frequency and intensity of magnetic field is noted. Differences observed between control and experimental specimens from one experiment to the next may possibly result from rhythms with lower frequencies that could not be investigated within the restraints of time available and hence, until proof is offered to the contrary, can not be viewed as simply statistical or procedural artifacts. Results from Experiment 7, in which pinnules were exposed to electric fields of different intensities (but with no imposed magnetic field), are also compromised by changes in the control group. These changes are probably due to deterioration in the condition of pinnules during the 91 hours of in vitro study.

Physical deterioration of the pinnules was very evident during exposure to combined magnetic and electric fields in Experiments 5 and 6. As a consequence,

the movement of the pinnules was inhibited, resulting in an increased rhythm mesor (pinnules tending to remain unfolded) and decreased amplitude of the circadian movement rhythm. The passage of current through copper plates may be suspected of causing the deterioration because exposure to a similar electric field applied between stainless steel plates (Experiment 7) did not result in such obvious physical deterioration.

In Experiment 9 there was no evidence of change in control pinnules, other than a possible slight shift in the rhythm's acrophase, during the 86 hours of study. The passage of a 300 pA, 60 Hz current through the stem joining the pinnules did, however, result in a reduced mesor (smaller average pinnule-angle), while the amplitude and acrophase of the rhythm resembled control values. In other words, the extent of rhythmic pinnule movement did not change but the average angle about which this movement occurred was smaller. The leaflets were, on the average, in a more folded condition throughout a 24-hour span than when the current was not applied. In the absence of a confirming experiment, further consideration of this effect seems inappropriate.

The differences observed between effects of a 0.4-gauss and a 1-gauss field (both in the presence of an electric field) on pinnules in vitro--experiment 12--also deserve further study.

III) Studies on an insect: Tribolium confusum (flour beetle).

The flour beetle exhibits a circadian rhythm in sensitivity to an organophosphate insecticide, dichlorvos, which acts upon the nervous system. Experiments were performed to determined whether or not the presence of an ELF (magnetic) field might accentuate the insecticide's toxicity, and whether any such synergistic effect may depend on the circadian stage at which beetles are exposed to the insecticide.

1. Methods.

Insects used in these studies were obtained from a colony maintained in the Department of Entomology, Fisheries and Wildlife, University of Minnesota. They were reared in a room kept at 25 ± 1°C and with lights on from 06^{00} to 18^{00} daily.

The insects in the experimental group were exposed to a 1 gauss magnetic field for 7 days before treating with the insecticide. The field was applied with a system of 3 coils energized by a Hewlett-Packard 200 CD function generator and a Bogen CHB35A amplifier. On the day of insecticide treatment (when all insects tested were 21 days old) a 1% (wt/vol) stock solution of dichlorvos in acetone was prepared. Appropriate dilutions for a dose-response test were made from this stock solution. For both experimental and control (not field-exposed) groups, separate subgroups of 400 insects were treated with insecticide at one of 4 different timepoints (03^{30}, 09^{30}, 15^{30} and 21^{30}) during a single 24-hour span. Four insecticide concentrations were used and 4 replicates of 25 adult insects were tested at each concentration. Insects were removed from the food medium 2 hours before testing and were exposed to the insecticide at 25 ± 1°C.

Insecticide solution (0.5 ml) was distributed uniformly over an onionskin paper lining a glass petri dish (9.0 cm dismeter). The solvent was allowed to evaporate completely for 6 minutes. Insects were introduced into the center of the treated surface. After 6 minutes they were removed and transferred to a second dish containing food medium. Following treatment with the insecticide, exposure to the magnetic field was continued under the original conditions. The number of dead insects was counted one week after treatment. Failure of the beetles to respond by movement to slight pressure exerted with a dissecting needle was the criterion used to determine death. Three such experiments (A, B and C)

were performed on three different batches of beetles. The frequency of the 1 gauss field was 60 Hz in studies A and B and 75 Hz in study C.

2. Results.

Table 3 presents the computed concentrations (in micrograms dichlorvos insecticide per square cm surface) required for 50% mortality--LC_{50}, along with the slope of the regression lines, for the 4 treatment times in each of the 3 experiments.

Considering overall mean values, the LC_{50} was greatest (and thus the susceptibility of insects least) if the beetles were exposed to the insecticide at 09^{30}, whether or not a magnetic field was applied. This result is consistent with previous information on the circadian susceptibility rhythm. Although the LC_{50} values tended to be slightly lower for field-exposed insects--suggesting a greater sensitivity to the insecticide--the differences were not significant, due to the rather large variation among the 3 experiments. Differences in slope of the dose-response regression lines, between control and field-exposed beetles, also were not significant.

We conclude that under these test conditions adult flour beetles showed no effect of an ELF 1-gauss field on susceptibility to dichlorvos.

IV) Studies on a mammal: Mus musculus (mouse).

Circadian rhythms are well-documented in representatives of this species (7, 10, 11). Prominent examples are rhythms in body temperature and in susceptibility to drugs.

A) The susceptibility of mice to a cardioactive drug, ouabain, in the presence and absence of an ELF electric field.

These investigations were performed to test the hypothesis that effects

of a field may be more evident if an animal is severely challenged by a toxic drug. Because the susceptibility of mice to a variety of harmful agents exhibits a circadian rhythm it is possible that any such synergistic effects of the field and drug may also vary with time. Accordingly, the response of mice to ouabain, a cardioactive drug, in the presence and absence of an electric field, was determined at defined circadian stages.

Three experiments were performed, the first 2 involving exposure to a 45 Hz, 136 v/m field and the third using a 75 Hz, 25 v/m field.

1) Methods and results, experiments 1 and 2.

Male BALB/cCr mice, approximately 13 months of age, were subjects in the first study, and female BALB/c mice, approximately 5 months of age, were used in the second. All mice were individually housed in a room maintained at 24 ± 2°C, with lighting from 06^{00} to 18^{00} alternating with darkness from 18^{00} to 06^{00} for about one week prior to the administration of ouabain. Food and water were freely available. Animals in the experimental group were continuously subjected to a 45 Hz electric field during this week.

The electric field was generated by an HP Model 200 CD wide-range oscillator, the voltage of which (26V) was applied to two stainless steel plates (25 x 50 cm) 19 cm apart. The plastic cage with the singly housed animals was placed between these plates. The whole setup was placed on stainless steel racks which were grounded with the oscillator. The electric field in the cage was therefore quite inhomogeneous. Since, however, the presence of the animal distorts the field, and since many investigators have indicated that a homogeneous field might have no or at least a lesser effect upon experimental animals than an inhomogeneous field this was regarded as an advantage, rather than a shortcoming, of the design.

In the first study the drug was administered to separate subgroups of mice at two circadian stages (defined in terms of the lighting regimen, a

known synchronizer of circadian rhythms): about 2 hours after lights-on (AM) and 2 hours after lights--off (PM). Three different dosages of ouabain were used at each time-point for both experimental and control groups. Following injection, exposure to the electric field was continued for half of the experimental mice. Figure 12 presents results from the PM time-point of the first study, as percent mortality at the different ouabain dosages for the control and field-exposed animals, regardless of whether the field exposure continued after ouabain injection. Although a chi-square test does not indicate statistical significance, the fact that the field-exposed mice had lower mortality at all three dosages is interesting. There was no consistent difference between control and field-exposed mice challenged with ouabain at the AM time-point. This is understandable in view of earlier studies (12, 13) showing that susceptibility of mice to ouabain is highest during the morning hours; any small influence of fields may therefore be completely obscured.

The second study focused on the PM time-point (about 4 hours after lights-off) and employed a single dosage of ouabain. This was administered to 50 mice, 30 of which had been exposed to the 45 Hz field continually during the previous week. The results are presented in the following table:

Table 4: Mortality of female BALB/c mice after injection with ouabain, 12 mg/kg, i.p., with and without exposure to a 45 Hz electric field.

Treatment	No. dead	No. surviving	% dead	X^2
No field (controls)	9	11	45	.003 ($P > .05$)
45 Hz electric field	12	18	40	

Although again the difference in mortality between the control and experimental groups was not statistically significant, the field-exposed mice had a slightly lower susceptibility, in keeping with the first study.

In summary, we can say with some confidence that the exposure of our mice to a 45 Hz electric field continuously for about one week prior to, as well as after, ouabain injection did not increase their susceptibility to this drug. In fact, the presence of such a field may have decreased susceptibility to a slight extent.

2) Methods and results, experiment 3

Female C57 BL/J6 mice were housed 5/cage at a room temperature of 26 ± 3°C, with lights on daily from 07^{00} to 19^{00} and with food and water freely available. After 2 weeks of standardization under these conditions, about half of the mice were subjected to a continuous 75 Hz electric field at 25 volts/meter between the metal cage top and a metal plate just beneath the plastic cage.

After the experimental group had been exposed to a 75 Hz, 25 v/m field for three weeks, ouabain was administered i.p. to separate subgroups of control and field-exposed mice at 4-hr intervals during a single 24-hr span and mortality was recorded.

Unfortunately, the dosage of ouabain (selected on the basis of a preliminary dose-response investigation) proved to be too low so a change was made to a higher dosage for the last 3 time points of the 24-hr study.

Table 5 presents the results of the entire 24-hr study. There was no statistically significant difference in mortality between control and field-exposed mice at any of the injection times, separately analyzed, nor in the overall totals. Applying the chi-square test to data from only the last 3 timepoints, for which the same ouabain dosage was used, gave $\chi^2 = 1.03$, $P > .05$, again not supporting a field effect. On the other hand, it should be noted that, in keeping with the first 2 experiments (above), mortality was slightly lower in the field-exposed mice, suggesting again the possibility of a small favorable field effect.

Disregarding the results from the first three time points because of improper dosage, data from the last three timepoints for both control and field-exposed mice

are consistent with the previously-demonstrated circadian rhythm in response to ouabain, insofar as mortality was significantly lower during the early dark span (21^{00}) than during the mid-light (13^{00}): $X^2 = 13.47$, $P < .05$.

IV. B. Changes in the body weight of young mice during a span of several months in the presence or absence of a 75 Hz, 1 gauss magnetic field.

This study was conducted under the premise that, if magnetic fields with characteristics similar to those in the vicinity of project-Sanguine antennae are injurious to health, we might expect to see an effect on body weight, a "global" index of general well-being.

1. METHODS

Female Bagg albino (BALB/c) mice, 4 weeks old (weanlings) were grouped 8 per plastic cage and assigned to one of two study-groups (I and II) housed in two environmental chambers, with artificial lighting from 07^{00} to 19^{00} and darkness from 19^{00} to 07^{00}. Food and water were continuously available.

Although both chambers were controlled at 75°F and 50% relative humidity, the two study groups were exchanged between chambers at regular intervals to ensure that any effects of subtle chamber differences would be equalized.

Both chambers were equipped with 2 coils about 35 cm apart and connected in series, each coil consisting of 19 turns of #14 magnet wire on a rectangular form 90 x 115 cm. The coils in the chamber containing group II mice were energized with a Hewlett-Packard 200 CD function generator feeding a 75 Hz sine wave into Bogen CHB35A Amplifiers to produce a uniform 1-gauss field in the chamber. The coils in the chamber containing group I, on the other hand, were <u>not</u> energized, as a control for any induction-effects.

Two separate but similar investigations were carried out. In the first (Study A), involving 94 mice (48 in group I, 46 in group II), group I was inadvertently subjected to the magnetic field and group II was removed from the field, for 2 weeks beginning when the animals were about 6 weeks old. No such mixup occurred in the second investigation (Study B), involving 48 mice in each group. Study B began 1.5 months after the start of Study A. The body weight of all mice was determined monthly, at the mid-light stage of the circadian rhythm.

2. RESULTS

Table 6 summarizes data obtained during a 4.5-month span in the case of Study A and a 3-month span for Study B. There was no consistent difference in body weight between control animals and those exposed to the magnetic field continually for several months.

IV. C. Circadian Temperature Rhythm and Other Variables in Mice Exposed to Combined 75Hz Magnetic and Electric Fields.

Investigation of possible field effects on mice described thus far (IV. A. and B.) tested electric and magnetic fields separately, under a variety of conditions as summarized in the following table:

Study	Field	Frequency Hz	Intensity	Span of Field Exposure	Variable Examined
1 & 2	Electric	45	136 v/m	1 week	Susceptibility to ouabain
3	"	75	25 v/m	3 weeks	"
4	Magnetic	75	1 gauss	3-4½ months	Body weight change

The remainder of this final report concerns the concurrent exposure of mice to a magnetic field and to an electric field plus a simulated "ground current"--all at 75 Hz. Two intensities of electric field--1 & 10 v/m--and three inten-

sities of magnetic field--0.5, 1& 2 gauss--were tested. We examined the characteristics of the circadian temperature rhythm (an index of general health) before and during prolonged exposure to different field combinations. Body weight and food consumption were also monitored.

1) Methods

One hundred and eight female BALB/c mice were randomly allocated, with stratification by age and weight, to 12 groups of 9 mice each. At an age of about 9 weeks, 36 of these animals (3 from each group) had temperature transensors implanted intraperitoneally (14). One implanted mouse and two unimplanted mice from the same group were housed together in each of 36 plastic cages with living space about 15 x 15 x 12 cm. San-i-cel bedding was present in all cages. Twenty-four of these cages (2 from each group) had floor "grids" positioned just above the bedding. The grid consisted of 25 stainless steel bars, 15 cm long, spaced about 0.6 cm apart on 2 plexiglass end-frames and connected with 2400-ohm resistors. With this grid, voltage gradients could be applied to simulate conditions at the Sanguine site. A rectangular stainless steel plate, about 15 x 12 cm, was attached vertically to each of the terminal bars of the grid so that mice also could be exposed to electric fields in their living space. When the mice were about 15 weeks old, one group (3 cages) was placed on each of 3 shelves in each of 4 environmental chambers (American Instrument Co.). Each cage was put on an antenna (within an RF-shield) for reception of information on body temperature of the transensor-bearing mouse. Each of the 4 chambers had 2 coils around its interior to permit the application of magnetic fields. These coils, spaced about 35 cm apart and connected in series, each consisted of 19 turns of #14 magnet wire on a rectangular form about 90 x 115 cm.

The chambers were maintained at 24 ± 3°C, about 50% relative humidity, and with lights on daily from 12^{00} to 24^{00}. Food (Purina Rat Chow) and water were freely available. Clean cages, grids, bedding and water bottles were provided at weekly intervals.

No fields were applied during the animals' first week of residence in the chambers. After this control span for all 4 chambers one chamber continued without a magnetic field while mice in the second, third and fourth chambers were exposed to 75 Hz magnetic fields of 0.5, 1 and 2 gauss, respectively. Also beginning with the second week, 75 Hz voltages were applied to grids of cages on 2 of the shelves in each chamber, 1 v/m (RMS) to those on one shelf and 10 v/m to those on another shelf. The fields and voltages were generated with a Hewlett-Packard 200 CD function generator feeding a 75 Hz sine wave into Bogen CHB35A amplifiers.

Thus, from the second week onward there were 12 combinations of 75 Hz magnetic and electric fields ranging from zero gauss, zero v/m to 2 gauss, 10 v/m--a different combination for each of the 12 groups of mice. To equalize exposure of different experimental groups to possible subtle environmental difference among and within chambers, the groups with their respective field combinations were rotated among chambers or shelves at weekly intervals. Body temperature data were automatically recorded on magnetic tape at 10 minute intervals, and body weights and food consumption were determined weekly.

2) Results

a) Body weight and Food Consumption

Table 7 summarizes the gain in body weight of unimplanted mice during the 4 weeks after combined electric and magnetic fields were turned on.

An analysis of variance indicated no statistically-significant difference among the 12 means in this table ($F = 1.64$; 11, 36 df; $P > .05$) and thus no

effects of these electric and magnetic fields on body weight.

Although one implanted mouse died during the span of field-exposure, the mean body weight gain of transensor-bearing mice (1.37 g) was actually greater than that of all unimplanted mice (1.00 g)---$t = 2.43$ with 105 df; $p < .02$. The presence of the transensor (weighing about 4 g) was apparently well-tolerated so we consider the telemetered temperature data as representative of the unencumbered mouse.

The results in Table 7 were of course obtained from mice maintained on grids, with which the electric fields were applied (see Methods). However, the mean body weight gain of these animals (1.07 g) was not significantly different from that of control unimplanted animals not living on grids (0.86 g)---$t = 1.35$, 70 df; $p > .05$. Thus, we conclude that the presence of grids had no adverse effect.

In keeping with the similarity of body weight gain among the 12 groups of mice exposed to the various field combinations (Table 7), there was no statistically significant difference in food consumption among them ($F = 1.87$; 11, 12 df; $p > .05$).

b) Circadian temperature rhythm

Temperature data, obtained from transensor-bearing mice by telemetry, were first summarized for each animal in the form of a "serial section", a plot of temperature data and characteristics of the circadian temperature rhythm day-by-day. Figure 13 is such a serial section summarizing data obtained from a mouse during the five weeks of the study. Units of the abscissal scale are in days. Units for the ordinate scales, one for each row of the figure, are presented on the right-hand side of the figure. The top row shows hourly averages of temperature values. In the second (narrow) row are plotted P-values indicating the adequacy of a 24-hour cosine model used to assess characteristics of the circadian rhythm. A P-value $\lesssim .05$ is considered satisfactory. In the third and fourth row are plotted daily estimates of circadian rhythm parameters obtained from the least-squares fitting of a 24-hour cosine curve (9). The bottom curve in the third row presents

estimates of the rhythm's Mesor, in this case the average temperature throughout a 24-hour cycle. The distance between a point on this bottom curve and a point directly above it on the upper curve indicates an estimate of the rhythm's Amplitude; i.e., half the peak-trough excursion.

Daily estimates of the rhythm's Acrophase (timing of the high point of the fitted cosine in relation to an arbitrary point on the 24-hour clock) are shown in the fourth row. The distance to dots above and below curves in rows 3 and 4 indicate the uncertainty of the respective estimates. Dashed vertical lines (event lines) indicate weekly cage cleaning and other changes: The first event line also indicates the time at which a 0.5 gauss magnetic field was applied to this particular mouse. This field was continued for the remainder of the record. The second, third and fourth events also indicate changes in location of the animal (see Methods).

This figure reveals stability of the circadian temperature rhythm in this mouse during the 5 weeks summarized. Fluctuations in the Mesor are probably related to the estrus cycle, since it is known that activity increases during estrus.

Figure 14 presents a similar summary in the case of an animal exposed to a 1 v/m electric field and 0.5 gauss magnetic field beginning after the first week. Of special importance is the absence of significant change in results at the time fields were turned on (first event line). The third and fourth weeks indicate gradual but marked changes in the rhythm's Mesor, Amplitude and Acrophase while results from the fifth week resemble those during weeks one and two. We have concluded that these variations are artifacts related to changes in the animals' location in the environmental chambers- moves that were made in the interest of equalizing environments of different groups (see Methods). Thus, this animal remained in a fixed location during weeks 1 and 2, was moved to a

different chamber at the start of week 3, then to a different shelf in the same chamber at the start of week 4 and finally was returned to its initial location starting with week 5. It is unlikely that a mouse would survive an actual body temperature elevation to about 112°F (indicated in top row) over the course of days. Furthermore, there was no evidence of loss in body weight or other indications of distress in this animal during weeks 3 and 4. As yet we are not able to explain the artifact completely although it appears that the presence of an electric field gradually alters the characteristics of the signal received from a transensor when the animal bearing it is moved among antennae of somewhat different design.

The fact that, among the 21 animals examined with such "serial sections", there was no discontinuity in the circadian rhythm at the time fields were turned on and that, with telemetry from the same location, there was no apparent difference in results between the first and fourth weeks of exposure to fields, leads us to conclude that the various 75 Hz field combinations studied had no significant effect on the temperature rhythm.

In support of this conclusion results from all mice with functioning transensors during the first, second and fourth weeks of this study are summarized in a series of "cosinor" plots, Figures 15 to 20.*

In these presentations time on the 24-hour scale is presented on the outermost two circles (in clock hours and in degrees, with $360^{o} = 24$ hours). The lighting regimen is indicated on the innermost circle; i.e., lights were on in the chambers from 12^{00} until 24^{00} each day. Each line directed outward from the center (i.e., each vector) represents animals studied under the field-conditions indicated at the top of the figure. The direction of this vector in relation to the circular

*Transensors functioned satisfactorily in 26 of the implanted mice during the first week in the chambers (without field). The number of functioning units fell to 23 by the second week and to 20 by week 5 (fourth week of exposure to fields).

scale indicates the acrophase, and its length the amplitude, of the circadian temperature rhythm. Circles or ellipses surrounding the tip of each vector show the 95% confidence region for these estimates. Results, including the rhythm-Mesor, are also presented numerically in a table at the bottom of the figure and as bar graphs at the right.

In Figures 15 to 17 results are presented for mice grouped according to their exposure to electric fields (and "ground currents")—0, 1 or 10 v/m—disregarding the presence of magnetic fields. Figure 15 represents the first week of the study (before fields were turned on), Figure 16 the first week in which mice were exposed to fields and Figure 17 the fourth week of field-exposure. The fact that all vectors are grouped, with overlap of 95% confidence regions, indicates that the presence of these electric fields had no statistically-significant effect on the acrophase and amplitude of the temperature rhythm. Mesors (overall average temperatures) were also essentially identical with or without the fields.

In Figures 18 to 20 mice are grouped according to magnetic-field exposures (0, 0.5, 1 or 2 gauss) regardless of electric fields, Figure 18 representing the first week of the study (without fields), Figure 19 the first week with exposure to fields and Figure 20 the fourth week of field-exposure. Although group C (all mice exposed to the 1-gauss field) was poorly represented by a cosine curve (as indicated by a large 95% confidence region overlapping the center) we again conclude that there was no statistically-significant effect of these magnetic fields on the circadian temperature rhythm.

Figures 21 to 23 present results obtained from animals before and during exposure to three specific combinations of fields: 1 v/m plus 0.5 gauss (Figure 21), 10 v/m plus 0.5 gauss (Figure 22) and 10 v/m plus 2 gauss (Figure 23). In Figures 21 and 22 there is clearly no change in either amplitude or acrophase of

the temperature rhythm as a result of exposure to the respective field combinations. The apparent low amplitude during the week prior to the application of 10 v/m plus 2 gauss (vector A in Figure 23) is probably due to the poor fit of a 24-hour cosine curve to the data--as indicated by the 95% confidence region overlapping the center. However, vector B (representing the week of field-exposure) indicates that the temperature rhythm under this condition has practically the same acrophase and amplitude as those depicted in Figures 21 and 22 with or without field exposure.

c) Estrus Cycle

Vaginal smears were obtained at the time of terminating field exposure. The proportion of mice in the stages of estrus or proestrus are presented in Table 8, both in relation to electric field intensity (part a) and magnetic field strength (part b).

The results of a 2 x n X^2-test for differences in proportion are indicated in each case. No statistically significant effects of field exposure are revealed.

d) Survival

Following termination of field exposure all mice were removed from the environmental chambers and housed under routine conditions in our laboratory (on San-i-cel bedding in plastic cages, with food and water freely available, in a room controlled at 75°F and 50% relative humidity, and with room lighting for 12 hours daily). Transensors were removed (from 36 mice) and all animals were caged in the same grouping used during field exposure. The percentages of mice surviving in different treatment categories were recently determined, 14 months after termination of field exposure. Results for unimplanted mice are presented in Table 9.

There was no statistically significant effect of exposure to these 75 Hz magnetic or electric fields during young adulthood on survival at the time of this evaluation.

Considering other conditions of the study, we found that forcing the animals to live on a grid, with or without voltage, during the span of field exposure did not affect survival—46 of 72 mice maintained on grids and 16 of 36 control animals kept directly on bedding survived 14 months after ending field exposure: $X^2 = 0.42$; $P > .05$. On the other hand, the implantation, presence and removal of the transensor resulted in a statistically-significant reduction in survival 14 months after field termination and transensor removal. Only 40% of the mice which had transensors survived, while 81% of the unimplanted animals were still alive: $X^2 = 17.67$; $P < .01$. Although only a single death occurred at the time of the second surgery, we believe that trauma associated with repeated surgery, and not the presence of the transensor itself, is ultimately responsible for the increased mortality. The initial implantation procedure and the subsequeat added burden of the transensor undoubtedly must have had some effect on the animals. Nevertheless, our data on body weight gain (see above) and the fact that telemetered temperatures were in the range expected on the basis of information obtained by other means (rectal probes) provide assurance that our results on the circadian temperature rhythm are representative of the healthy intact mouse.

V) Conclusions.

These investigations into possible effects of ELF fields and currents on the silk tree, flour beetle and mouse had in common a consideration of circadian rhythms in the variables examined. By acknowledging that these variables (e.g., the susceptibility of an organism to a harmful agent) are predictably rhythmic

and that the characteristics of these rhythms may themselves change in response to ELF fields, we believe that the probability of detecting an effect was increased.

Of the many experiments conducted, some indicated a statistically significant effect of such fields. These findings were not observed consistently on repeated study. Some of these could, perhaps, be explained as the effect of rhythms with lower frequency, before they are regarded as artifacts of the methods used. In other cases, before we attribute the observation to statistical variation, it will have to be ascertained whether any effect may be transient. For example, in the investigation of possible effects of a 75 Hz, 1 gauss field on the body weight of mice (part IV. B., Table 6) analysis of data at one point (when mice in study A had been exposed to the field for 2.5 months and those in Study B 1 month) indicated a statistically-significant difference between control and field-exposed animals. Such a difference was not found in subsequent weighings of the same animals.

A few positive findings from in vitro studies of the silk tree--the effect of a 60 Hz stem-current on the overall extent of leaflet folding (Part II, experiment 9); the observation that a 0.4 gauss 60 Hz field may be more harmful than a 1 gauss 60 Hz field, in the presence of a 100 v/m electric field (Part II, experiment 12)--seem noteworthy and deserving of further study. In vivo investigations on this plant in the presence of a 1 gauss field (either alone--experiment 8--or in combination with a 100 v/m electric field and a 300 pA current--experiment 10) were clearly negative, however.

It should be noted that even the lowest field strengths tested (1 v/m and 0.4 gauss) exceeded those observed near the Sanguine antennae energized with 300 amps (15). It is conceivable that the endpoints examined are affected only at field intensities <u>below</u> those tested.

In summary, the many experiments described in this report--on plants, insects and mammals--have not yielded convincing evidence for an adverse effect of ELF magnetic and/or electric fields and currents.

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Table 1: SUMMARY OF EXPERIMENTAL DESIGNS FOR A. JULIBRISSIN*

Exp. No.	Condition of Pinnules	Days of standardization in LD 12:12**	No. of Pinnule Pairs (no. of plants)	Duration of data collection (hours) Pre-exposure	During exposure to fields	60 Hz Field v/m	gauss
1	in vitro	4	40 (2)	--	36	--	1
2	"	0	24 (2)	--	80	--	1
3	"	7 (LL)	24 (2)	--	107	--	1
4	"	3	24 (1)	49	29	--	1
5	"	10, 24	44 (2)	43	72	100	1, 0.5
6	"	15, 29	44 (2)	44	64	100	1
7	"	8	60 (2)	54	37	{0, 1, 10 100, 180}	--
8	in vivo	3	16 (1)	116	116	--	1
9	in vitro	10	60 (1)	40	46	(300 pA)	--
10	in vivo	7	32 (4)	172	305	{100 (+ 300 pA)}	1

*Experiments 11 and 12 not included in this table; see text.

**Prior to pinnule excision in the case of in vitro experiments

Table 2: PARAMETERS OF CIRCADIAN RHYTHM IN PINNULE MOVEMENT IN THE PRESENCE AND ABSENCE OF FIELDS

Summary of A. Julibrissin experiments; results from least squares (cosinor) analyses*

Pre-exposure							During Exposure to Field(s)				
				Amplitude					Amplitude		
Experiment	Group	n =	Mesor	Absolute	% of Mesor	Acrophase	n =	Mesor	Absolute	% of Mesor	Acrophase
1	C						20	42.7	28.3	76.8	-160
	E						20	50.3	31.2	55.1	-182
2	C						35	69.2	12.9	20.3	-239
	E						35	70.7	14.4	28.9	-205
3	C						60	81.7	34.1	41.9	- 39
	E						60	99.5	11.1	11.7	- 34
4	C	19	103.1	89.6	81.8	-197	20	131.0	28.4	21.7	-204
	E	19	104.6	78.9	82.0	-197	20	134.3	25.3	18.8	-212
5	C	18	99.4	84.5	85.2	-203	21	86.0	72.4	84.7	-205
	E	18	104.2	80.0	76.9	-195	21	134.1	11.1	8.2	-197
6	C	15	90.4	82.1	90.9	-199	22	97.1	71.3	75.8	-208
	E	15	90.2	82.0	90.9	-199	22	146.6	3.1	2.1	-243
7	0	18	74.8	92.1	123.2	-199	15	84.7	72.1	85.2	-188
	1	18	83.6	90.7	108.5	-193	15	84.1	69.9	83.1	-192
	10	18	82.4	93.2	113.0	-195	15	85.4	74.7	87.5	-192
	100	18	74.3	89.4	120.2	-195	15	76.8	80.7	105.1	-192
	180	18	88.9	95.4	107.3	-191	15	90.7	70.4	77.6	-191
8	C	39	81.5	86.2	105.8	-185	39	79.8	87.7	109.9	-201
	E	39	79.2	86.2	108.8	-176	39	78.6	85.2	108.4	-201
9	C	16	81.3	83.2	102.3	-217	19	82.6	85.2	103.1	-235
	E	16	81.1	83.4	102.8	-222	19	71.8	86.9	121.0	-236
10	C	67	72.1	85.9	119.1	-212	107	74.2	83.6	112.7	-212
	E	67	70.1	86.3	123.1	-211	107	71.9	86.0	119.6	-215

*Group C = controls, E = experimentals; for experiment 7 numbers in the Group column indicate voltage during exposure to fields; n = number of leaf-angle measurements analyzed; rhythm-parameters, Mesor, Amplitude and Acrophase were derived from least squares fitting of 24-hr cosine curve (see text); units of Mesor and Absolute Amplitude are in degrees of pinnule angle while units of acrophase are in degrees in relation to midnight (360° = 24 hrs).

Experiments 11 and 12 not included in this table; see text and figures 9, 10, and 11.

Table 1: SUSCEPTIBILITY OF FLOUR BEETLES TO DICHLORVOS AS A FUNCTION OF CIRCADIAN STAGE AND EXPOSURE TO 1-GAUSS ELF FIELD

Time of Dichlorvos Treatment[1]	Statistic[2]	Experiment (Hz)			
		A (60)	B (60)	C (75)	Overall Mean
a. Control beetles (no field).					
03^{30}	LC_{50}	18.6	20.1	17.8*	18.8
	Slope	5.28	7.51	7.69*	6.83
09^{30}	LC_{50}	25.4	24.6	17.3	22.4
	Slope	4.76	8.86	6.65	6.76
15^{30}	LC_{50}	20.2*	22.3	18.8	20.4
	Slope	5.06	7.95	8.73	7.25
21^{30}	LC_{50}	20.9	19.8	17.3	19.3
	Slope	4.95	10.56	6.96	7.49
b. Beetles exposed to field.					
03^{30}	LC_{50}	19.5*	20.1*	17.1	18.9
	Slope	4.26*	7.78*	8.28	6.77
09^{30}	LC_{50}	21.5	25.2	17.0	21.2
	Slope	6.85	5.20	7.00	6.35
15^{30}	LC_{50}	17.5	22.4*	17.4	19.1
	Slope	7.42	8.42*	6.72	7.52
21^{30}	LC_{50}	20.0	20.1	17.0	19.0
	Slope	5.29	9.83	8.79	7.97

*Non-significant regression.

[1] During exposure to magnetic field, before and after insecticide treatment, room lights were on from 06^{00} to 12^{00} daily.

[2] LC_{50} = concentration of dichlorvos (micrograms/cm^2) required for 50% mortality; Slope = slope of dose-response regression line; Each pair of values based upon 4 sets of 25 beetles exposed at each of four discrete concentrations of dichlorvos.

Table 5 : Mortality after ouabain injection at one of several different times during a 24-hr span, in control mice and in mice exposed to a 75 Hz, 25 v/m electric field.

Approximate Injection Time	Ouabain dose (mg/kg, i.p.)	Number dead/Total number injected		X^2	P
		Controls	Field-exposed		
01^{00}	3.0	4/39	8/42	0.64	>.05
05^{00}	3.0	1/43	3/44	0.24	"
09^{00}	3.5	13/43	14/44	0.01	"
13^{00}	4.0	32/43	27/44	1.15	"
17^{00}	"	31/43	29/43	0.06	"
21^{00}	"	17/42	15/41	0.02	"
TOTAL		98/253	96/258	0.07	"

Trble 6 : Body Weight Changes in Mice with and without Exposure to a 75 Hz, 1 gauss Magnetic Field

STUDY	Months of Field-Exposure:	BODY WEIGHT (Mean gm. ± SE) at 0 (weaning)	0.75	1.5	2.5	3.5	4.5
A	Group I (Control)	13.3 ± .2	18.5 ± .2	20.4 ± .2	21.2 ± .2	21.6 ± .3	22.7 ± .2
	Group II (Field-exposed)	13.3 ± .2	18.5 ± .2	19.8 ± .2	20.8 ± .3	21.5 ± .2	22.3 ± .2

	Months of Field-Exposure:	0 (weaning)	1	2	3
B	Group I (Control)	12.6 ± .2	18.0 ± .2	19.8 ± .2	21.4 ± .2
	Group II (Field-exposed)	12.6 ± .2	17.6 ± .2	20.1 ± .2	21.7 ± .2

Table 7: Changes in body weight of mice exposed to different combinations of magnetic and electric fields.

Electric Field (v/m) \ Magnetic Field (gauss):	Body Weight gain (g, mean ± SE)* during 4 weeks of exposure to: 0	0.5	1.0	2.0
0	1.55 ± .42	0.35 ± .50	1.33 ± .28	1.83 ± .37
1.0	1.23 ± .26	0.68 ± .31	1.05 ± .24	0.80 ± .14
10	0.83 ± .22	0.98 ± .25	1.35 ± .22	0.93 ± .39

*Each mean ± SE represents the 4 unimplanted mice housed in cages with grids, under each field combiations.

Table 9:

PROPORTIONS OF MICE IN ESTRUS OR PROESTRUS (E or PF) FOLLOWING EXPOSURE TO MAGNETIC FIELDS (PART a) OR ELECTRIC FIELDS (PART b).

a)	Magnetic Field (gauss)					
	0	0.5	1	2	X^2	P
Proportion in E or PE	18/27	17/25	13/26	11/26	5.06	>.05

b)	Electric Field (v/m)*				
	0	1	10	X^2	P
Proportion in E or PE	14/23	16/22	13/23	1.34	>.05

*Only mice maintained on grids during field exposure.

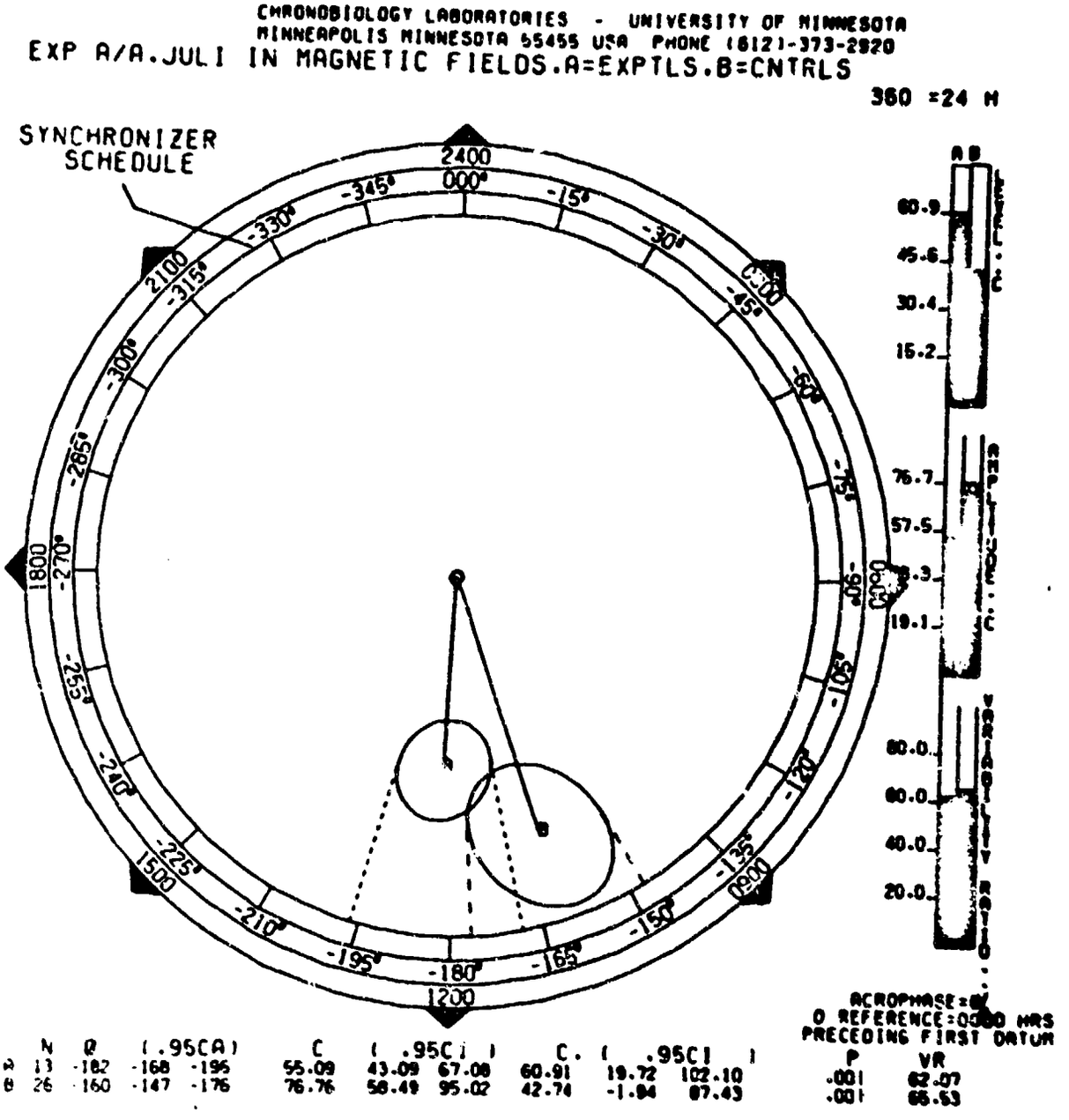
Table 9: PERCENTAGE OF UNIMPLANTED MICE SURVIVING, 14 MONTHS AFTER EXPOSURE TO MAGNETIC FIELDS (PART a) OR ELECTRIC FIELDS (PART b).

a)	Magnetic Field (gauss)					
	0	0.5	1.0	2.0	X^2	P
Percentage surviving:	72	82	88	83	1.57	>.05

b)	Electric Field (v/m)*				
	0	1	10	X^2	P
Percentage surviving:	60	87	81	3.28	>.05

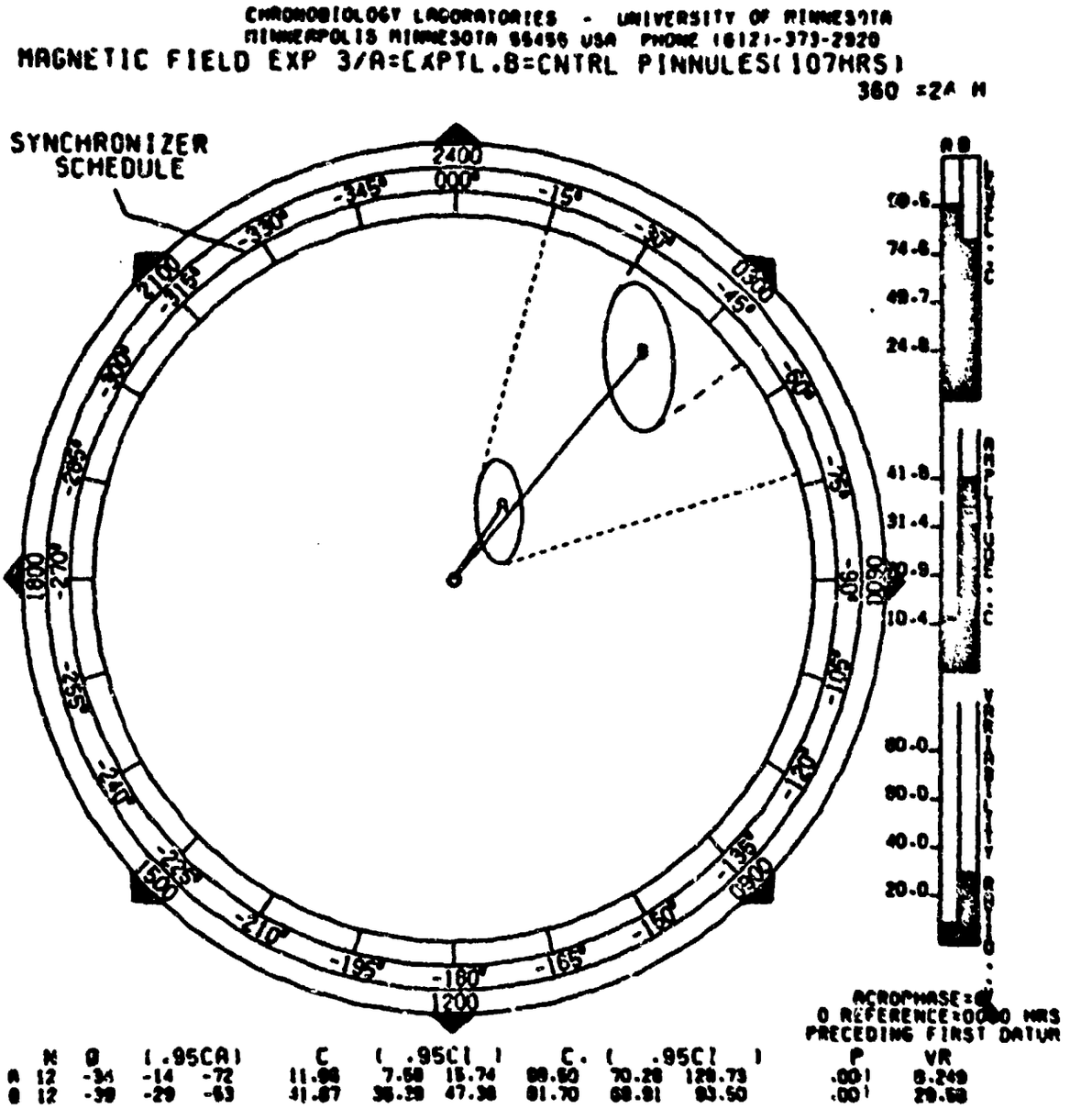
*Only mice maintained on grids during field exposure.

FIGURE 1



	N	R	(.95CA)		C	(.95CI)		C.	(.95CI)		P	VR
A	13	-182	-168	-195	55.09	43.09	67.08	60.91	19.72	102.10	.001	82.07
B	26	-160	-147	-176	76.76	58.49	95.02	42.74	-1.94	87.43	.001	65.53

FIGURE 2



	N	B	(.95CA)		C	(.95CI)		C.	(.95CI)		P	VR
A	12	-36	-14	-72	11.98	7.68	15.74	88.60	70.28	128.73	.001	8.249
B	12	-39	-29	-63	41.87	36.38	47.38	81.70	68.81	93.50	.001	29.68

FIGURE 3

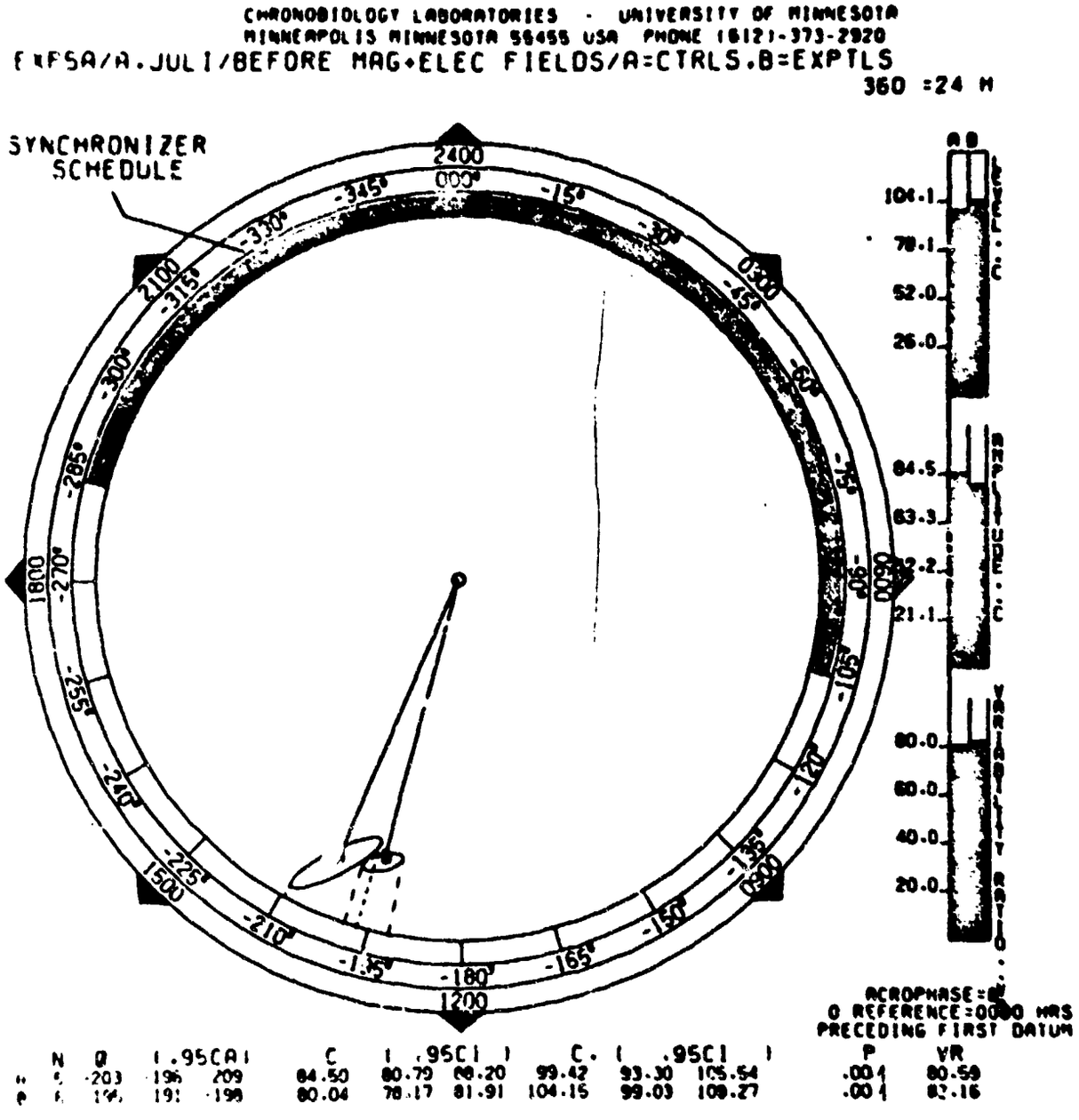
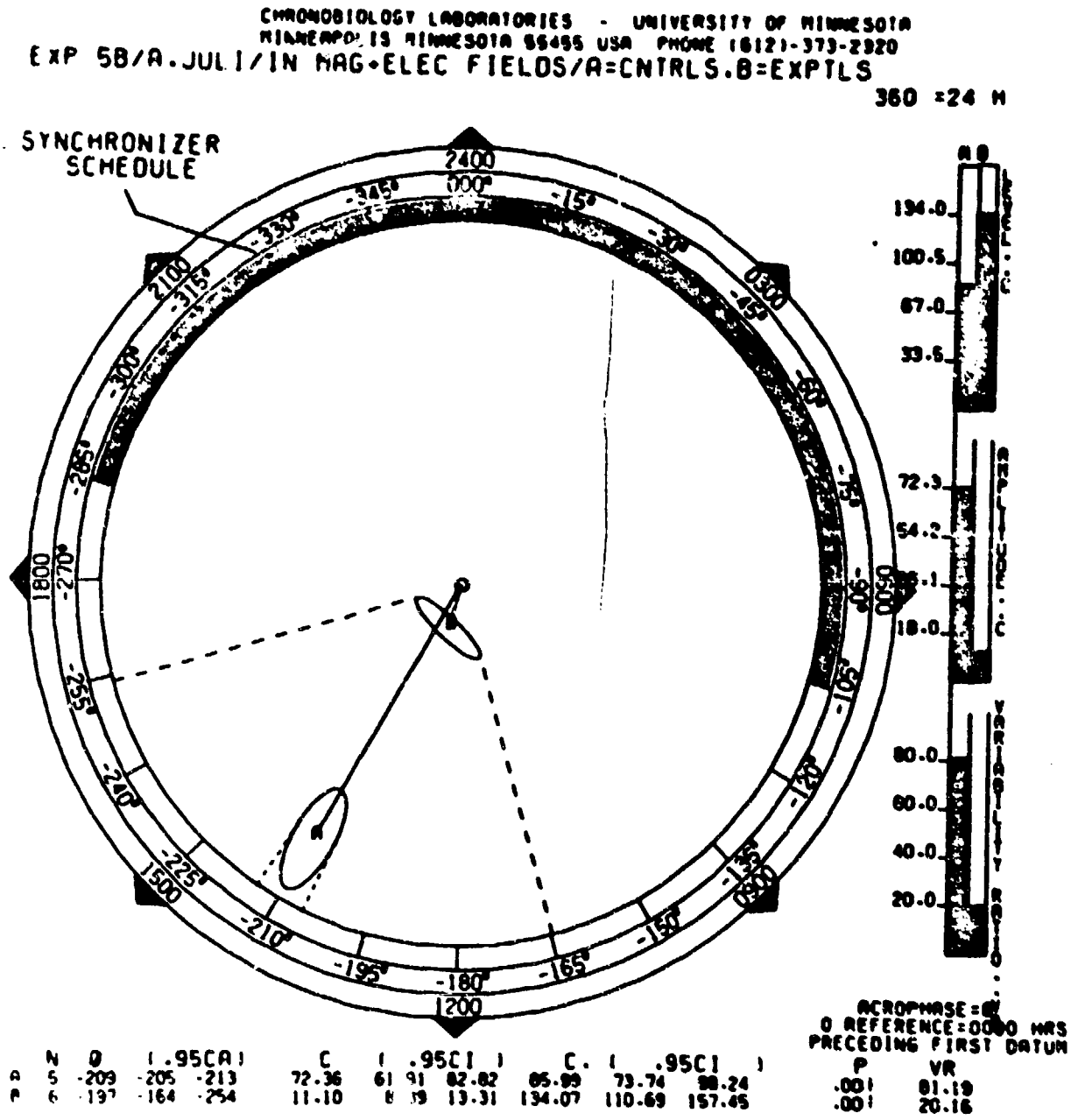
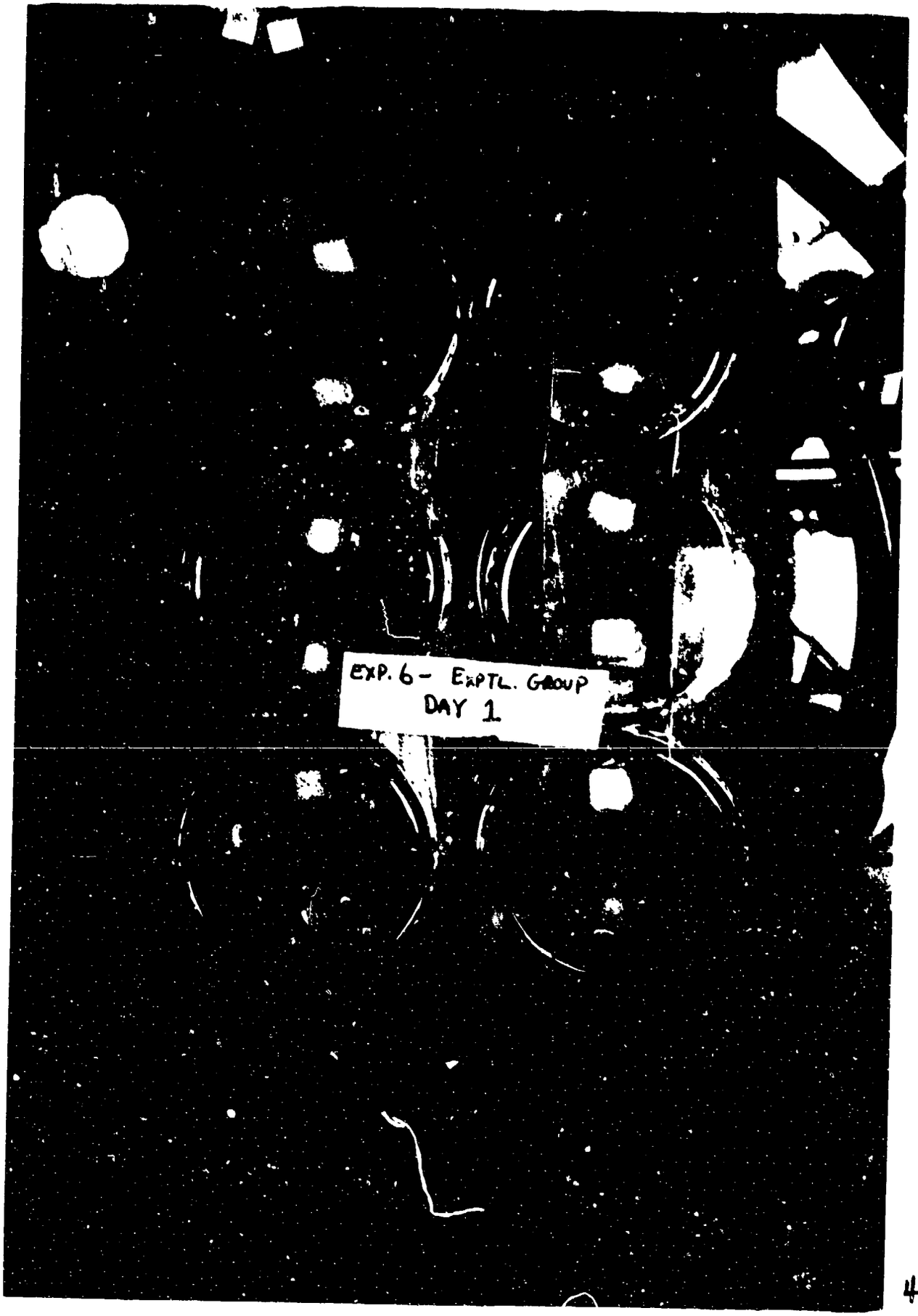

CHRONOBIOLOGY LABORATORIES - UNIVERSITY OF MINNESOTA
MINNEAPOLIS MINNESOTA 55455 USA PHONE (612)-373-2920
EXPSA/A.JUL1/BEFORE MAG+ELEC FIELDS/A=CTRLS.B=EXPTLS
360 =24 H
SYNCHRONIZER SCHEDULE
2400
000°
0300
0600
0900
1200
1500
1800
2100
-15°
-30°
-45°
-60°
-75°
-90°
-105°
-120°
-135°
-150°
-165°
-180°
-195°
-210°
-225°
-240°
-255°
-270°
-285°
-300°
-315°
-330°
-345°
104.1
78.1
52.0
26.0
84.5
83.3
82.2
21.1
80.0
60.0
40.0
20.0
ACROPHASE=
0 REFERENCE=00000 HRS
PRECEDING FIRST DATUM
N Ø I.95CI I C I .95CI I C. I .95CI I P VR
A 5 -203 196 209 84.50 80.79 88.20 99.42 93.30 105.54 .004 80.58
B 5 194 191 198 80.04 78.17 81.91 104.15 99.03 109.27 .004 82.16

FIGURE 4



	N	Ø	(.95CA)		C	(.95CI)		C.	(.95CI)		P	VR
A	5	-209	-205	-213	72.36	61.91	82.82	85.89	73.74	98.24	.001	81.19
P	6	-197	-164	-254	11.10	8.39	13.31	134.07	110.69	157.45	.001	20.16


EXP. 6 - EXPTL. GROUP
DAY 1

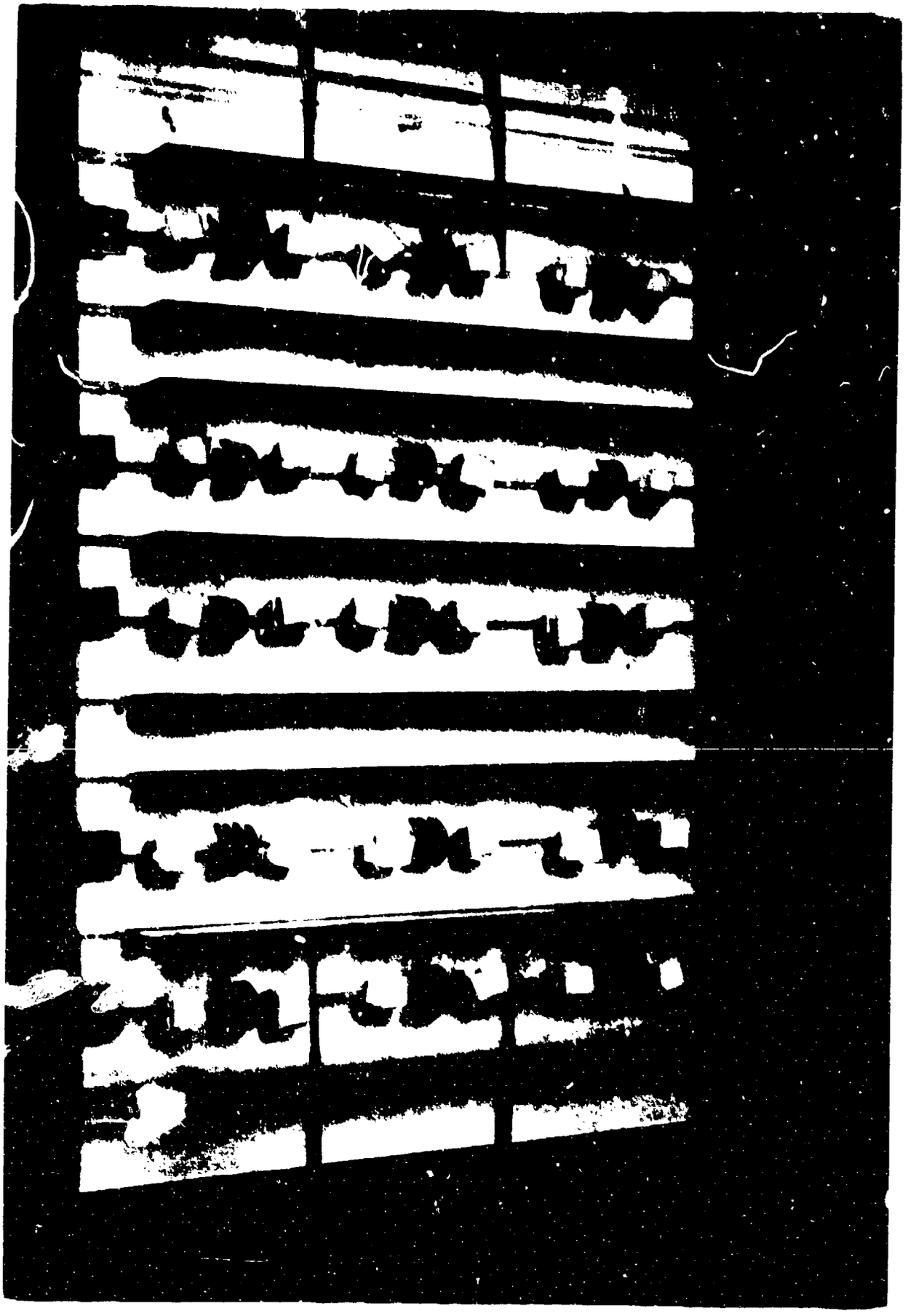


Figure 7

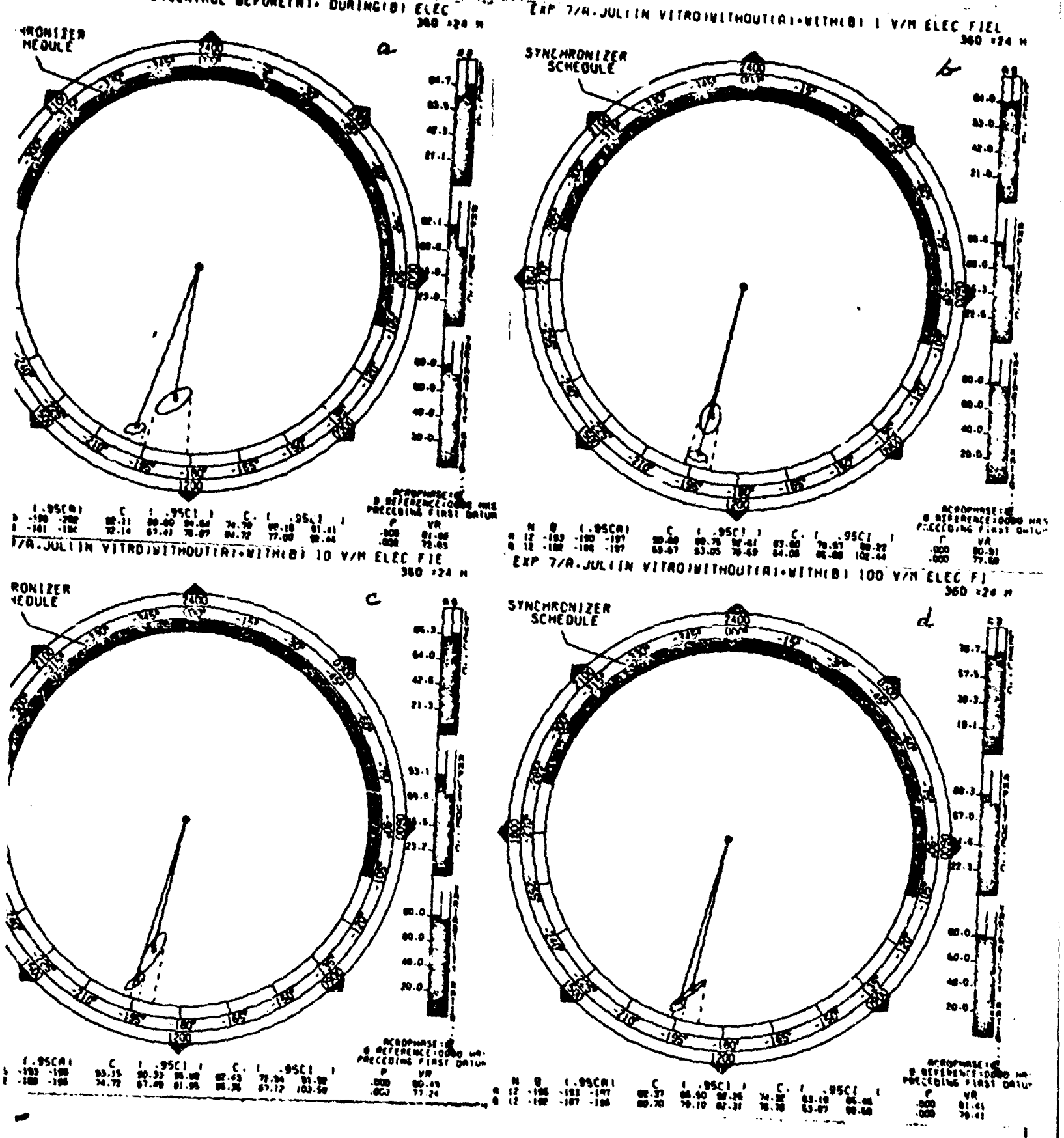

7/A.JULIN VITRO(CONTROL BEFORE(A)+ DURING(B) ELEC
SYNCHRONIZER SCHEDULE
a
EXP 7/A.JULIN VITRO(WITHOUT(A)+WITH(B) 1 V/M ELEC FIEL
SYNCHRONIZER SCHEDULE
b
7/A.JULIN VITRO(WITHOUT(A)+WITH(B) 10 V/M ELEC FIE
SYNCHRONIZER SCHEDULE
c
EXP 7/A.JULIN VITRO(WITHOUT(A)+WITH(B) 100 V/M ELEC FI
SYNCHRONIZER SCHEDULE
d

Figure 7e

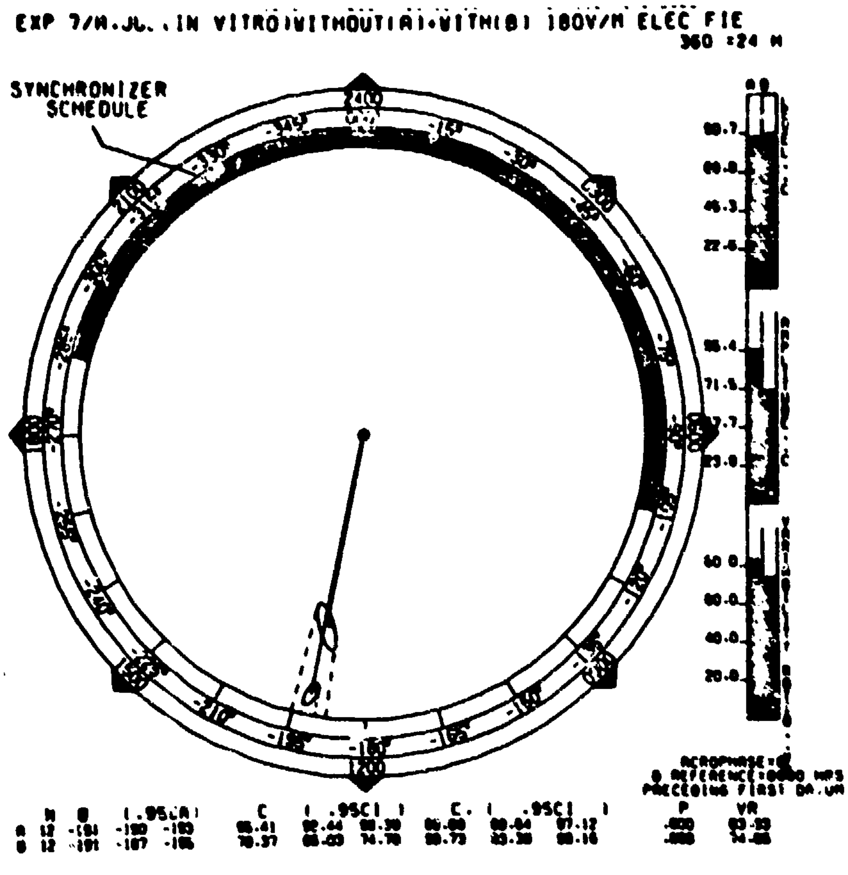
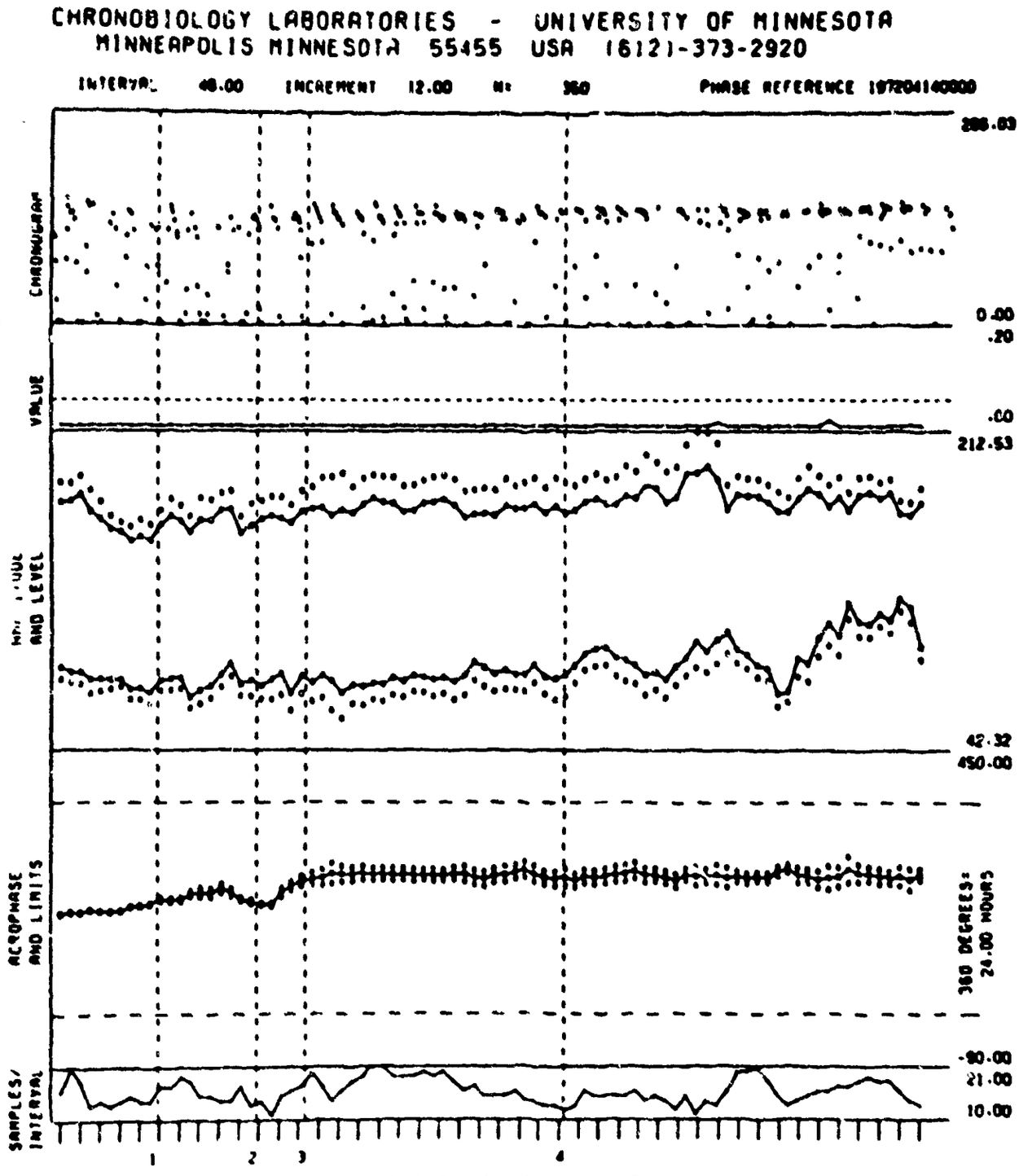

EXP 7/M.JL..IN VITRO(WITHOUT(A)+WITH(B) 180V/M ELEC FIE
360 =24 H
SYNCHRONIZER
SCHEDULE
2400
1200
ACROPHASE
REFERENCE
PRECEDING FIRST DA.UN
N
C
.95CI
P
VR

FIGURE 8



LEAF 2/MEAN OF 8 PINNULE ANGLES ON TWO PINNA

EXP A/ALBIZZIA JULI/IN VIVO/IN MAGNETIC FIELD + SHIFT

Fig. 9: Exp. II - A. Julibrissin Pinnules (In Vitro)

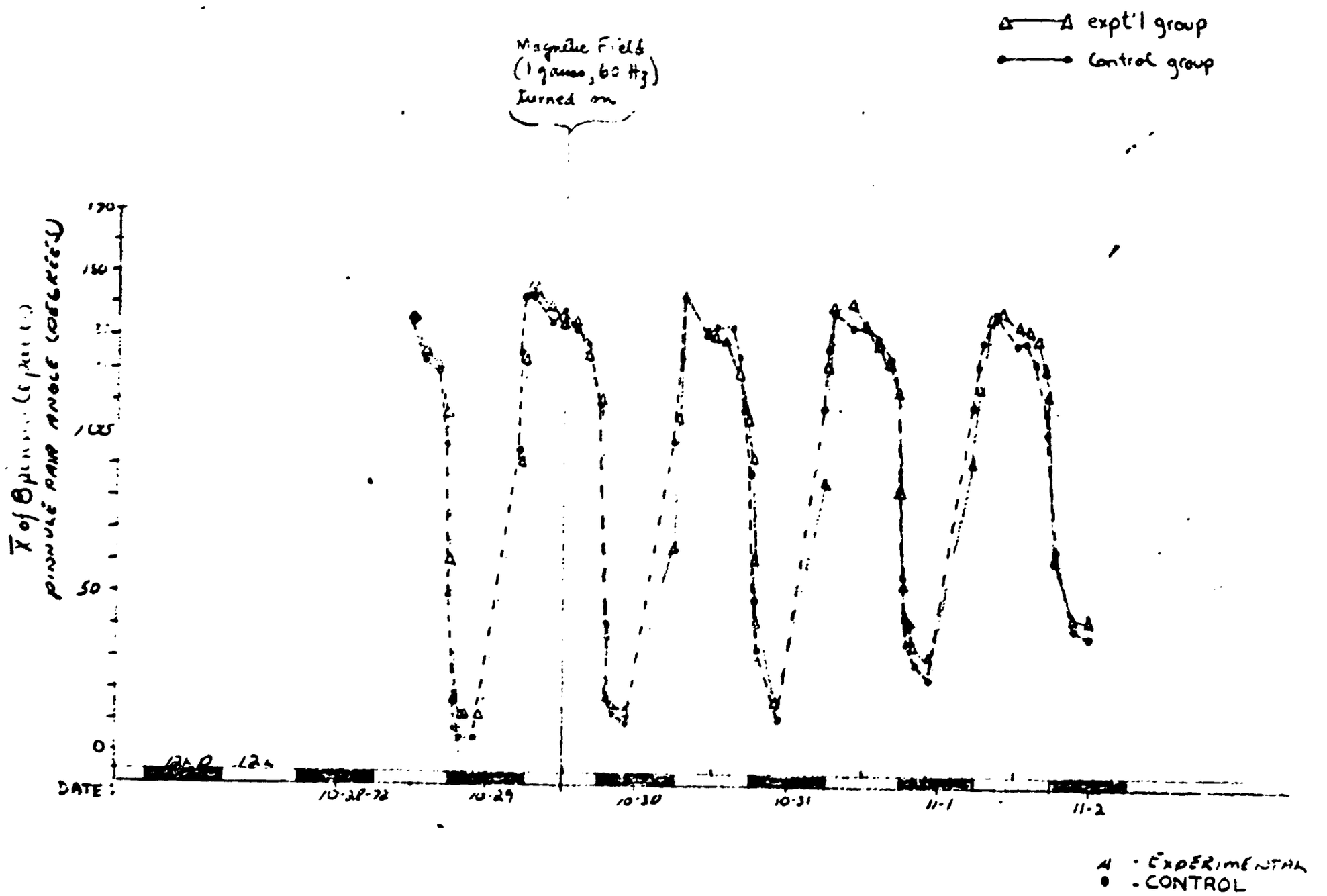


Fig. 9: Exp. II - A. Julibrissin Pinnules (In Vitro)

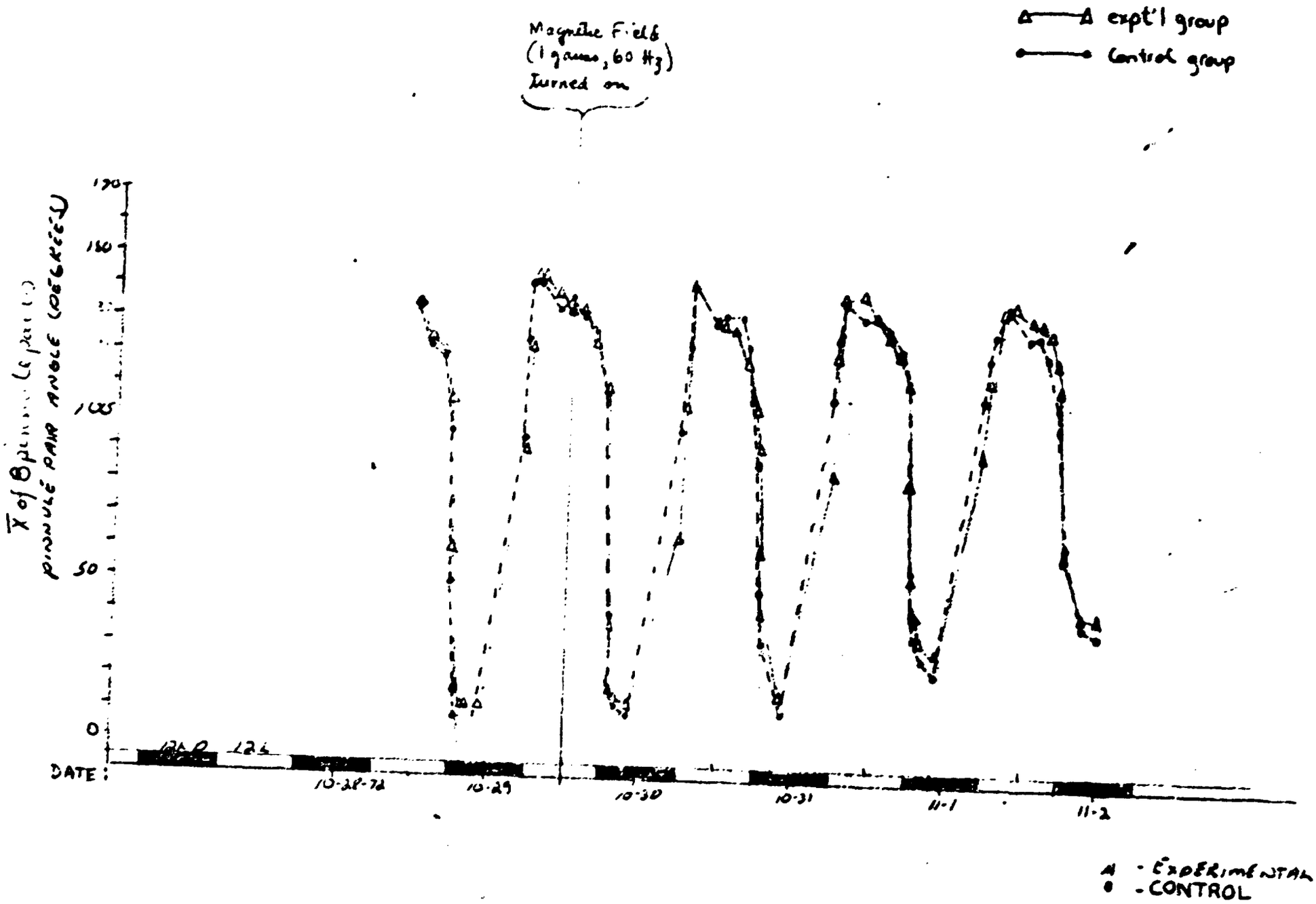


Fig. 10: EXP. II A. JULIBRISSIN (IN VIVO) MAGNETIC FIELD of 1 gauss (60 Hz)

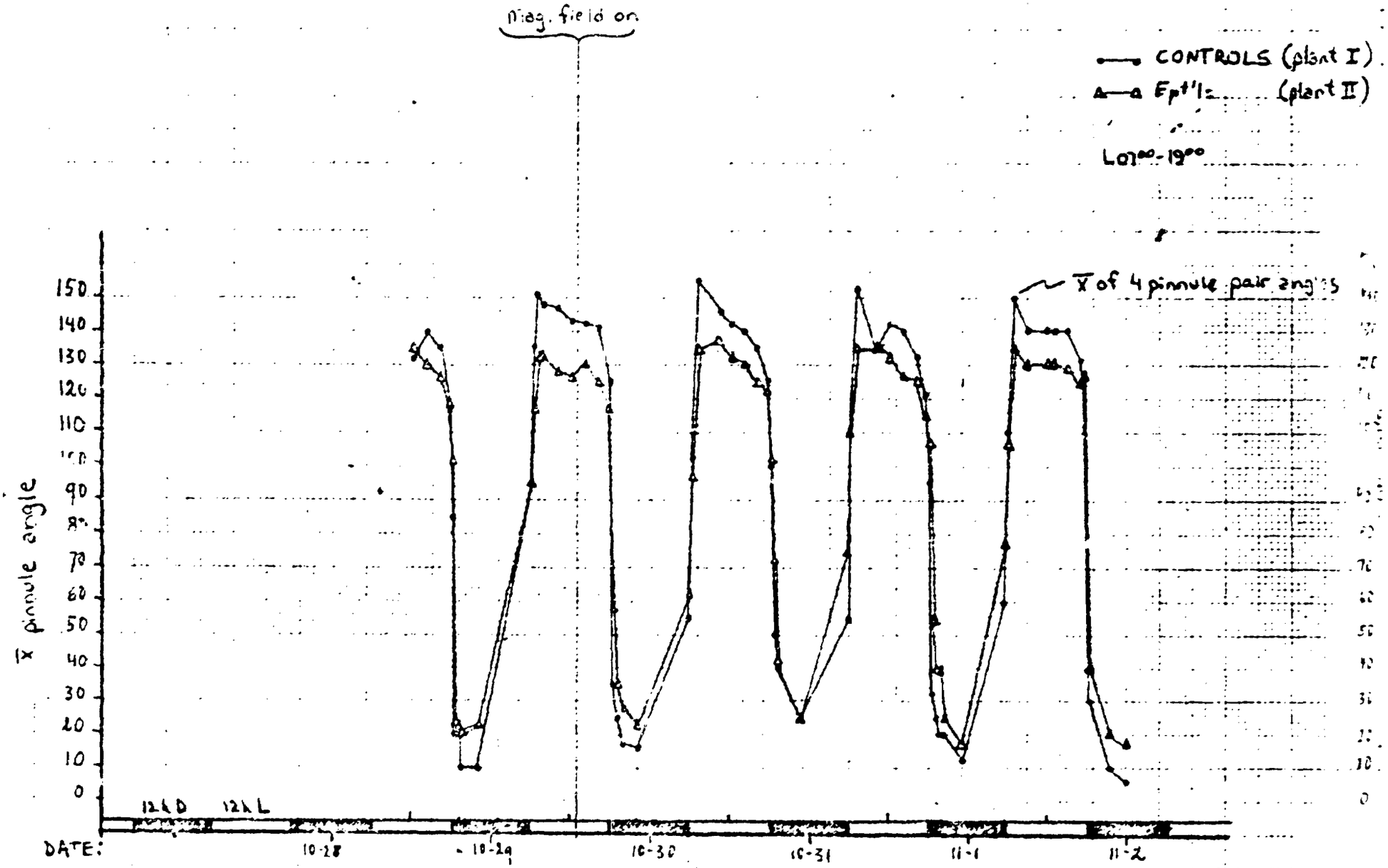


Fig 11 : EXP 12 ALBIZZIA JULIBRISSIN (PINNULES IN VITRO)

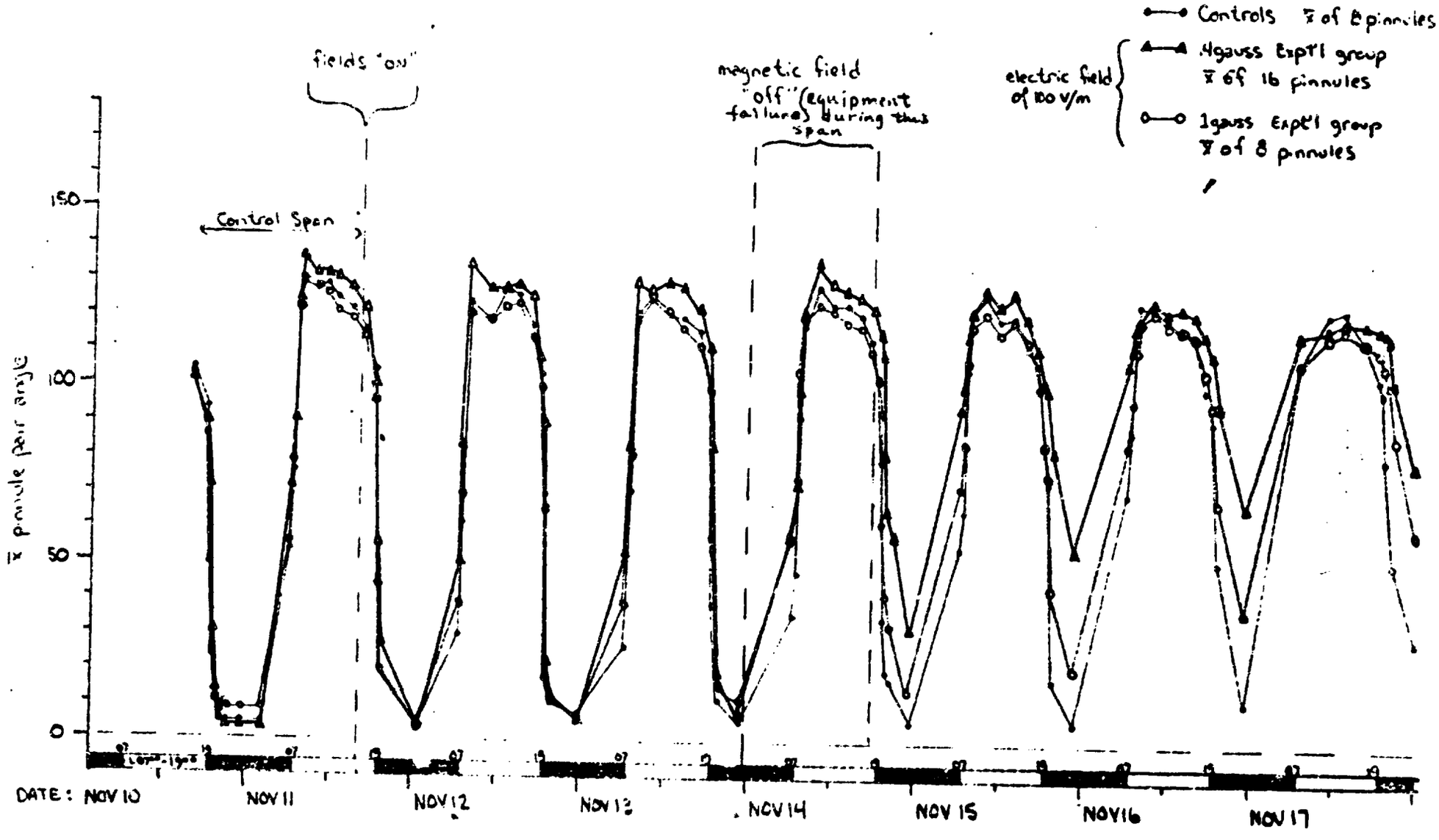


Fig 12: Does a 45 Hz, 136 V/m electric Field have an effect on susceptibility of mice to ouabain? 47<

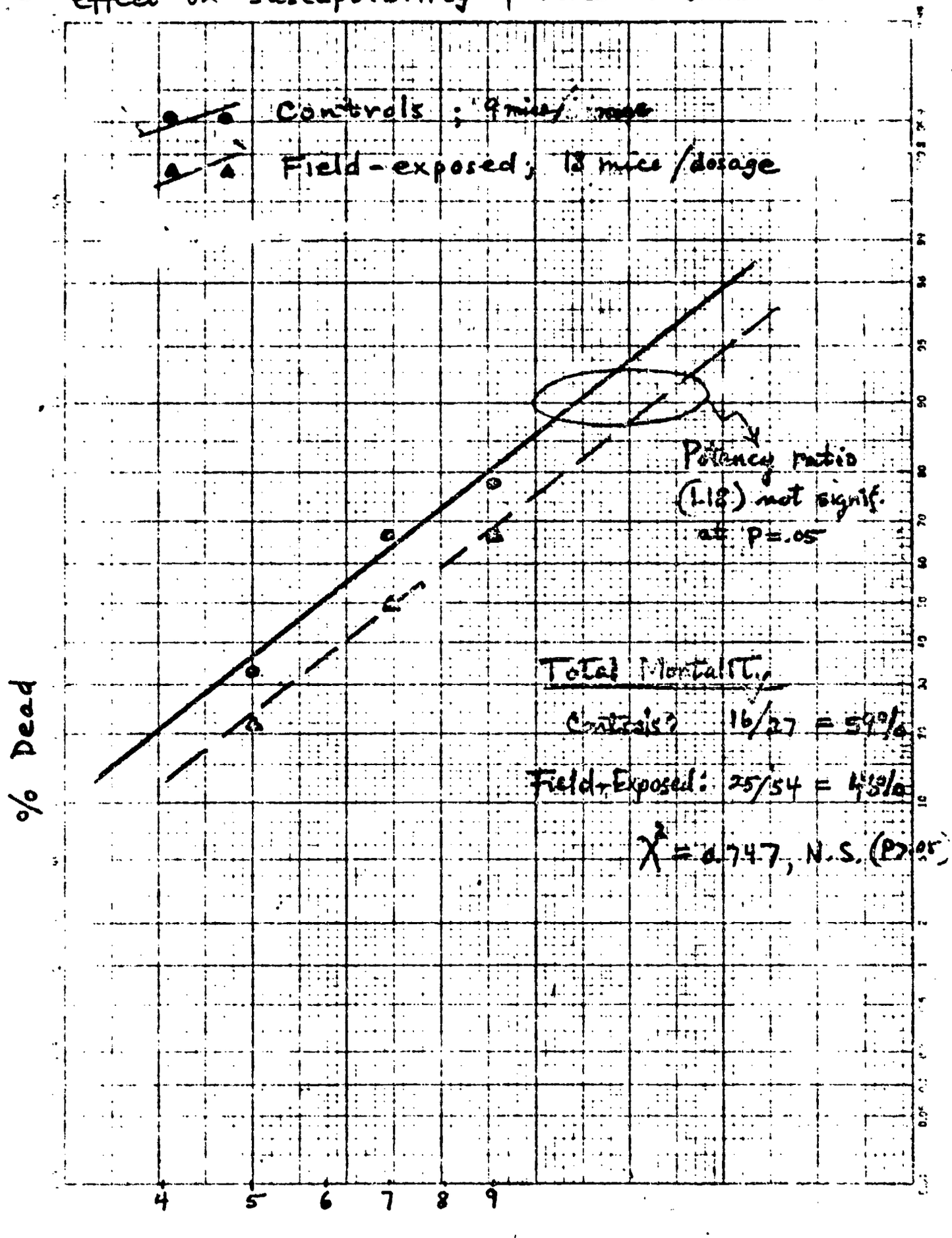


FIGURE 13

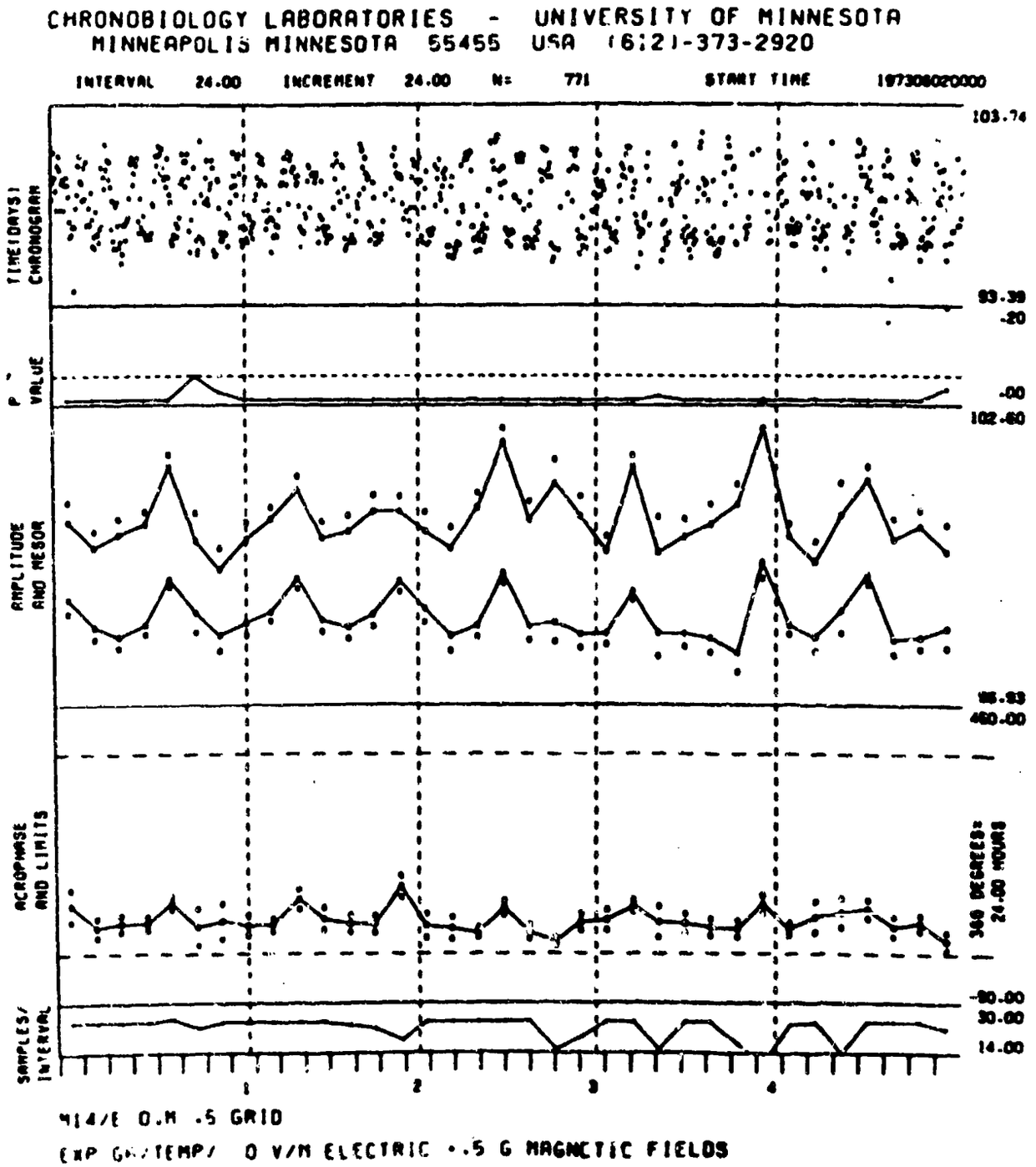

CHRONOBIOLOGY LABORATORIES - UNIVERSITY OF MINNESOTA
MINNEAPOLIS MINNESOTA 55455 USA (612)-373-2920
INTERVAL 24.00 INCREMENT 24.00 N= 771 START TIME 197308020000
TIMEIDAYS) CHRONOGRAM
103.74
93.38
.20
P VALUE
.00
102.60
AMPLITUDE AND MESOR
98.83
460.00
ACROPHASE AND LIMITS
360 DEGREES= 24.00 HOURS
-90.00
30.00
14.00
SAMPLES/ INTERVAL
1
2
3
4
414/E 0.M .5 GRID
EXP G/TEMP/ 0 V/M ELECTRIC .5 G MAGNETIC FIELDS

FIGURE 14

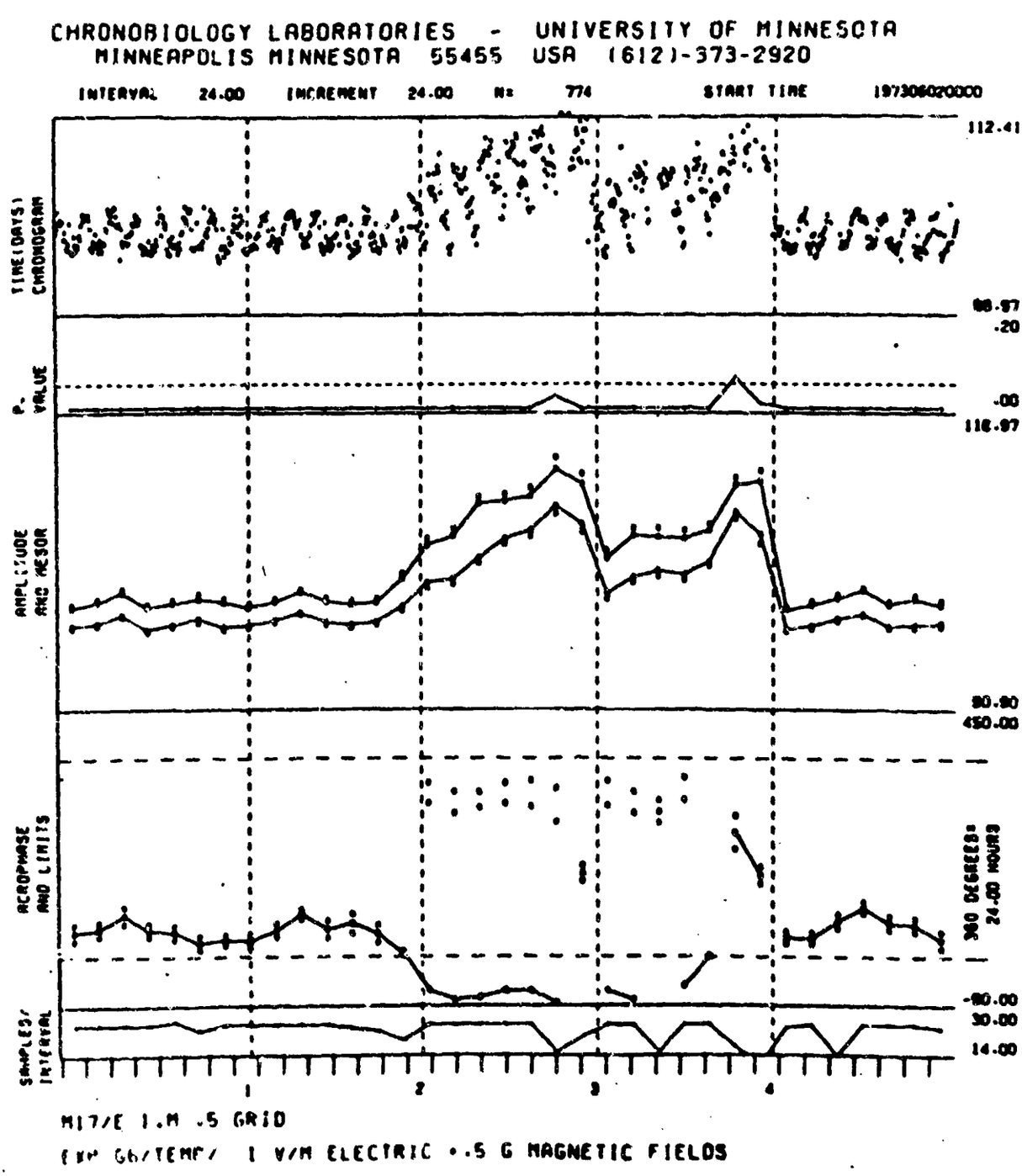
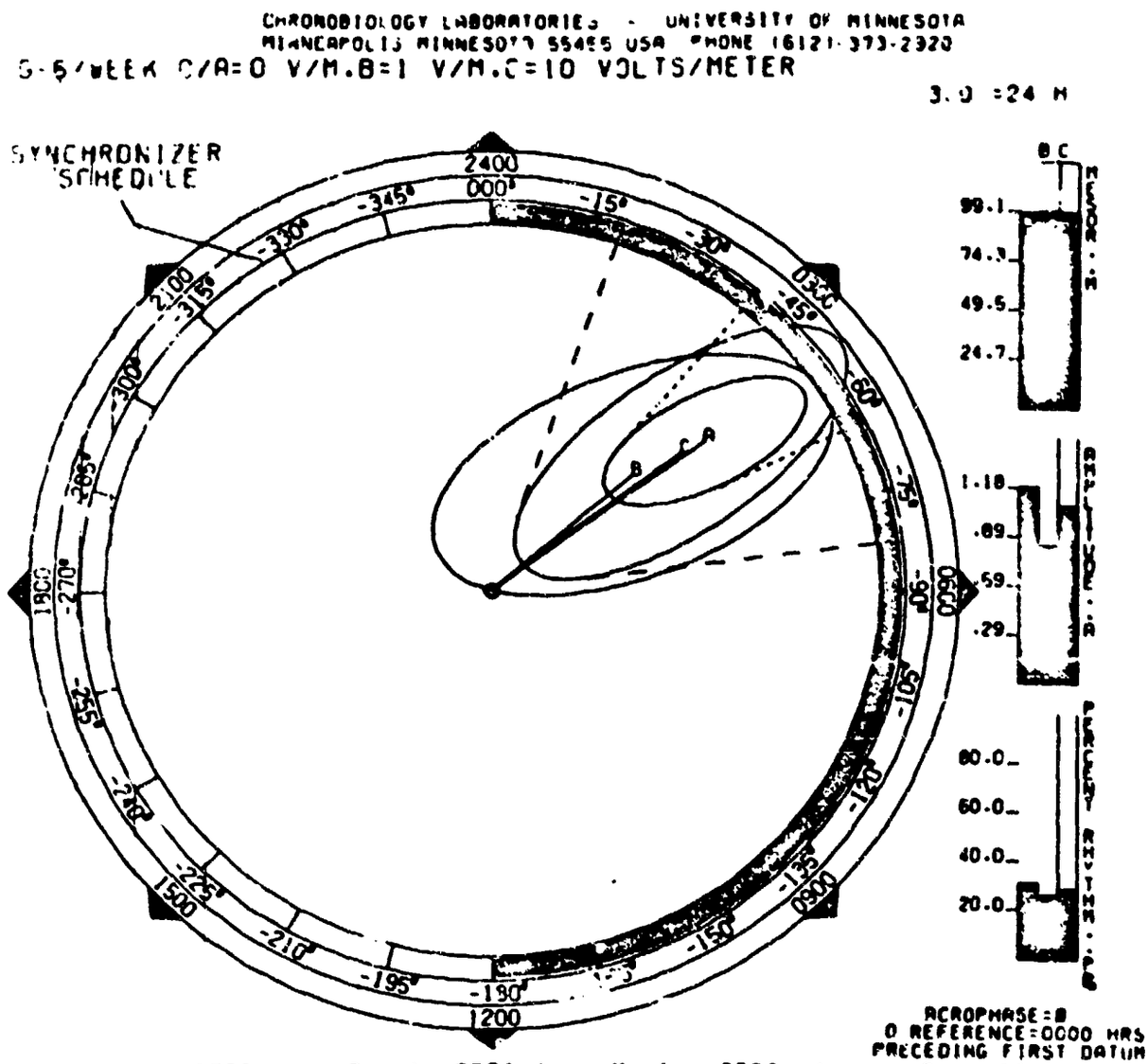

CHRONOBIOLOGY LABORATORIES - UNIVERSITY OF MINNESOTA
MINNEAPOLIS MINNESOTA 55455 USA (612)-373-2920
INTERVAL 24.00 INCREMENT 24.00 N= 774 START TIME 197306020000
TIME(DAYS) CHRONOGRAM
112.41
88.97
P. VALUE
.20
.00
116.97
AMPLITUDE AND MESOR
80.80
450.00
ACROPHASE AND LIMITS
360 DEGREES= 24.00 HOURS
-80.00
SAMPLES/ INTERVAL
50.00
14.00
1
2
3
4
MI7/E 1.M .5 GRID
EMF GB/TEMP/ 1 V/M ELECTRIC +.5 G MAGNETIC FIELDS

FIGURE 15



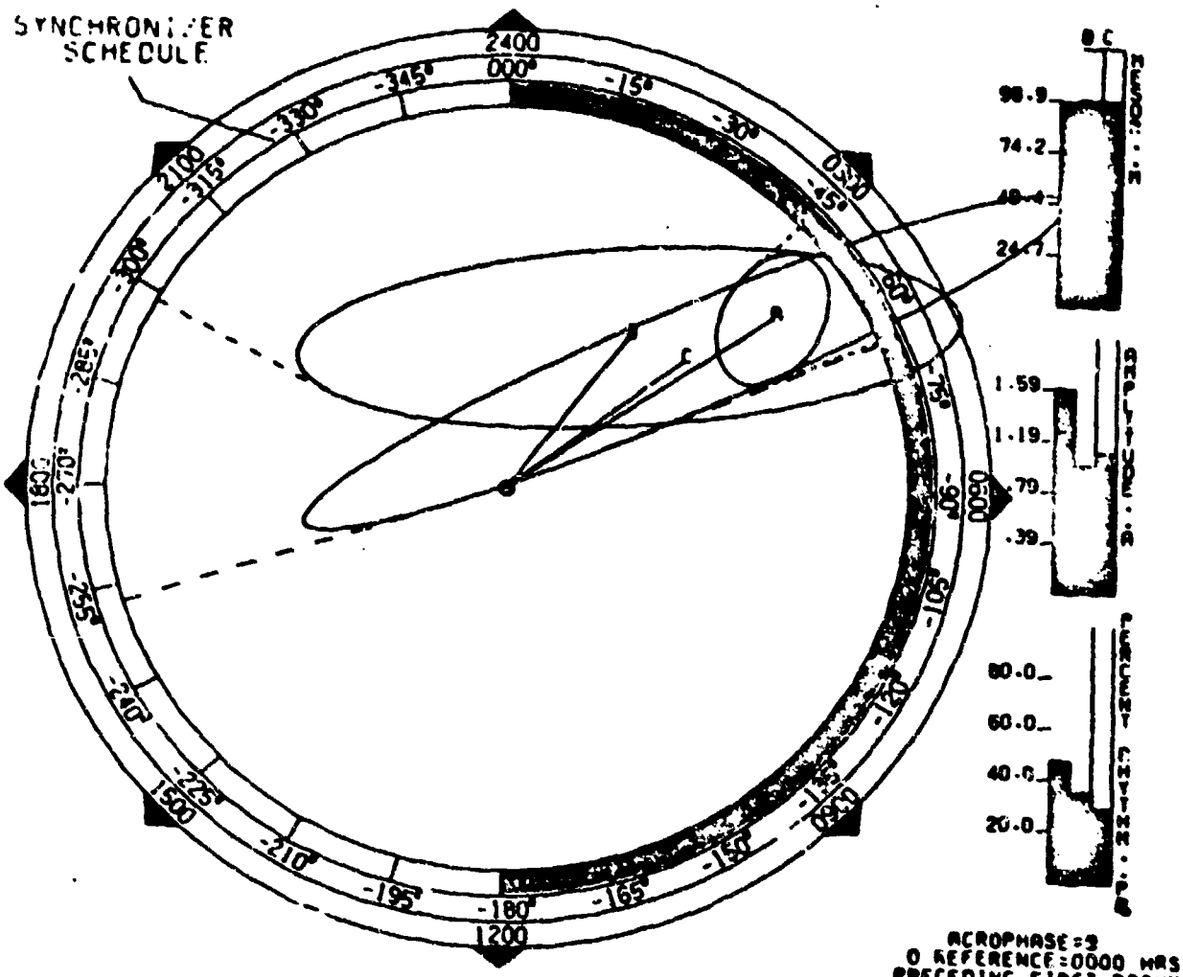
	N	Θ	(.95CR)		A	(.95CI)		M	(.95CI)		P	PR
A	5	-53	-40	-64	1.18	.78	1.58	99.08	57.34	100.82	.000	30.99
B	5	-48			.83	-.14	1.82	99.19	96.65	101.73	.050	26.33
C	5	-52	-18	-82	1.07	.01	2.12	99.01	96.92	101.10	.023	28.87

FIGURE 16

CHRONOBIOLOGY LABORATORIES - UNIVERSITY OF MINNESOTA
MINNEAPOLIS MINNESOTA 55455 USA PHONE (612)-373-2920

6/WEEK 1/A=0 V/M.B=1 V/M.C=10 VOLTS/METER

360 =24 H



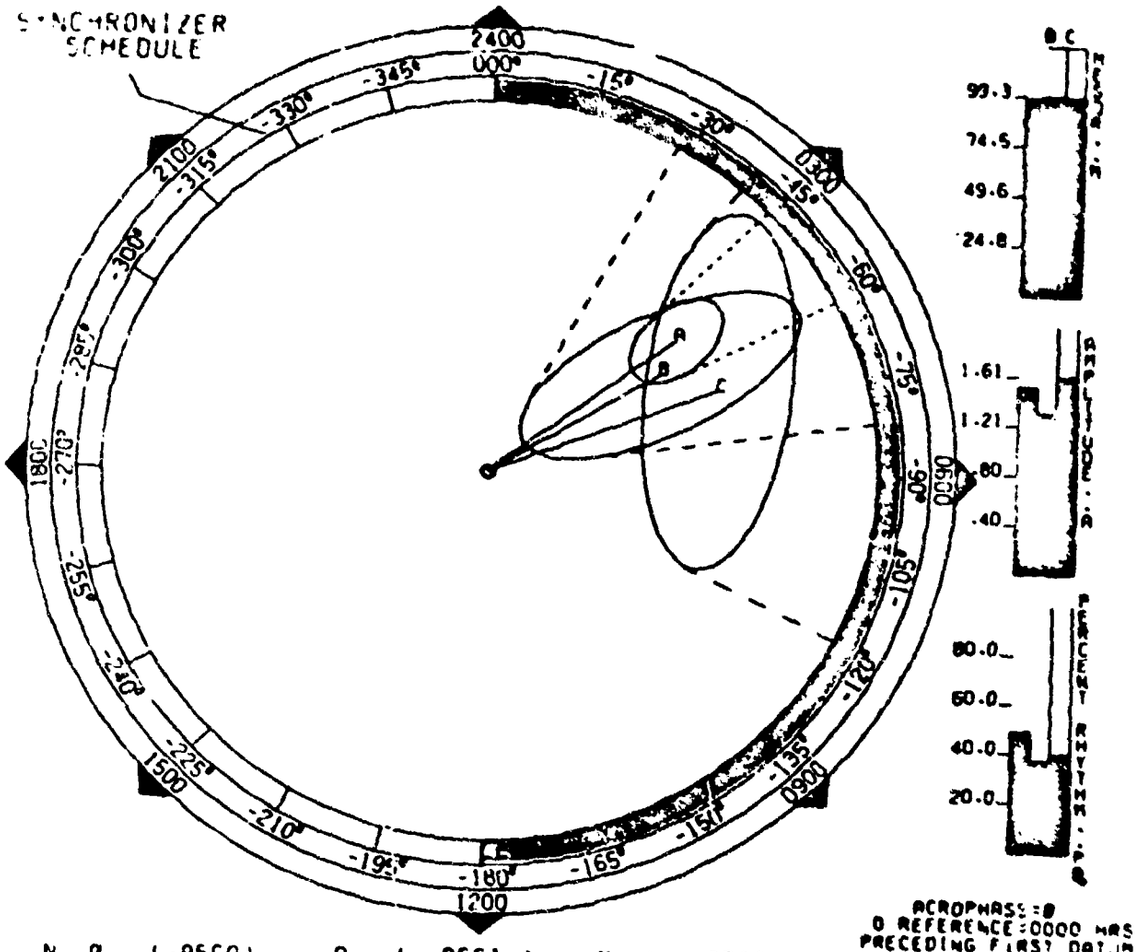
	N	Ø	(.95CI)		A	(.95CI)		M	(.95CI)		ACROPHASE=Ø REFERENCE=0000 HRS PRECEDING FIRST DATUM P	PR
A	8	-55	-46	-66	1.58	1.31	1.85	98.68	97.58	99.79	.000	47.87
B	7	-36	-72	-298	.99	.50	1.48	98.94	96.72	101.17	.008	35.42
C	5	-52	-67	-252	1.08	-.27	2.46	98.87	95.83	101.92	.045	28.98

FIGURE 17

CHRONOBIOLOGY LABORATORIES UNIVERSITY OF MINNESOTA
MINNEAPOLIS MINNESOTA 55455 USA PHONE (612) 373 2920

B/WEEK A/A=0 V/M.B=1 V/M.C=10 VOLTS/METER

350 =24 H



	N	Q	(.95CI)		A	(.95CI)		M	(.95CI)		P	PP
A	5	-53	-44	-61	1.53	1.25	1.80	98.49	97.54	99.44	.000	48.39
B	6	-58	-28	-81	1.31	.07	2.55	99.33	95.42	103.24	.020	37.68
C	5	-69	39	-116	1.61	1.20	2.01	99.18	96.03	102.33	.002	40.59

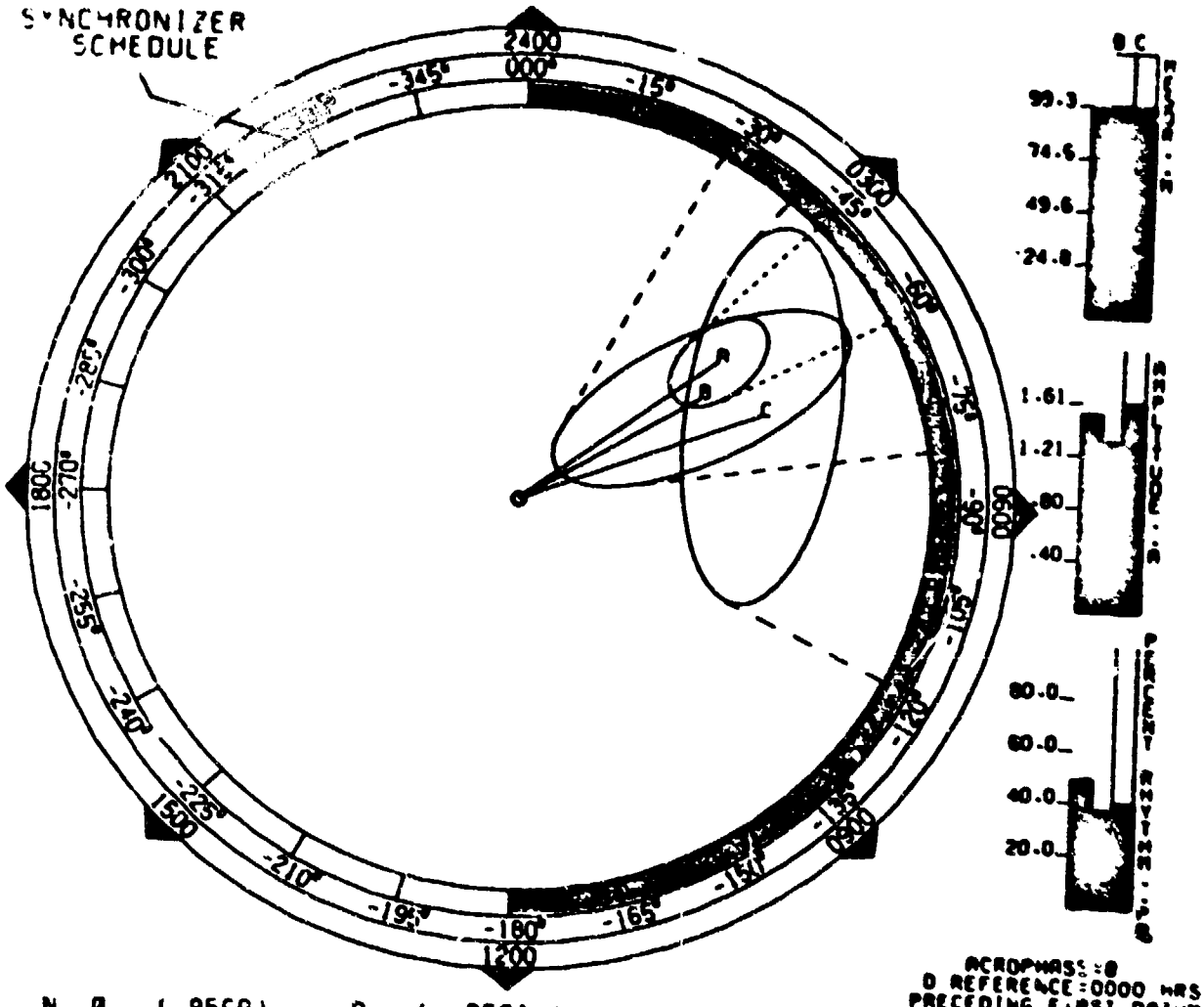
ACROPHASE=0
0 REFERENCE=0000 HRS
PRECEDING FIRST DATUM

FIGURE 17

CHRONOBIOLOGY LABORATORIES - UNIVERSITY OF MINNESOTA
MINNEAPOLIS MINNESOTA 55455 USA PHONE (612)-373-2920

6/WEEK A/A=0 V/M.B=1 V/M.C=10 VOLTS/METER

:00 :24 H



ACROPHASE:0
0 REFERENCE:0000 HRS
PRECEDING FIRST DATUM

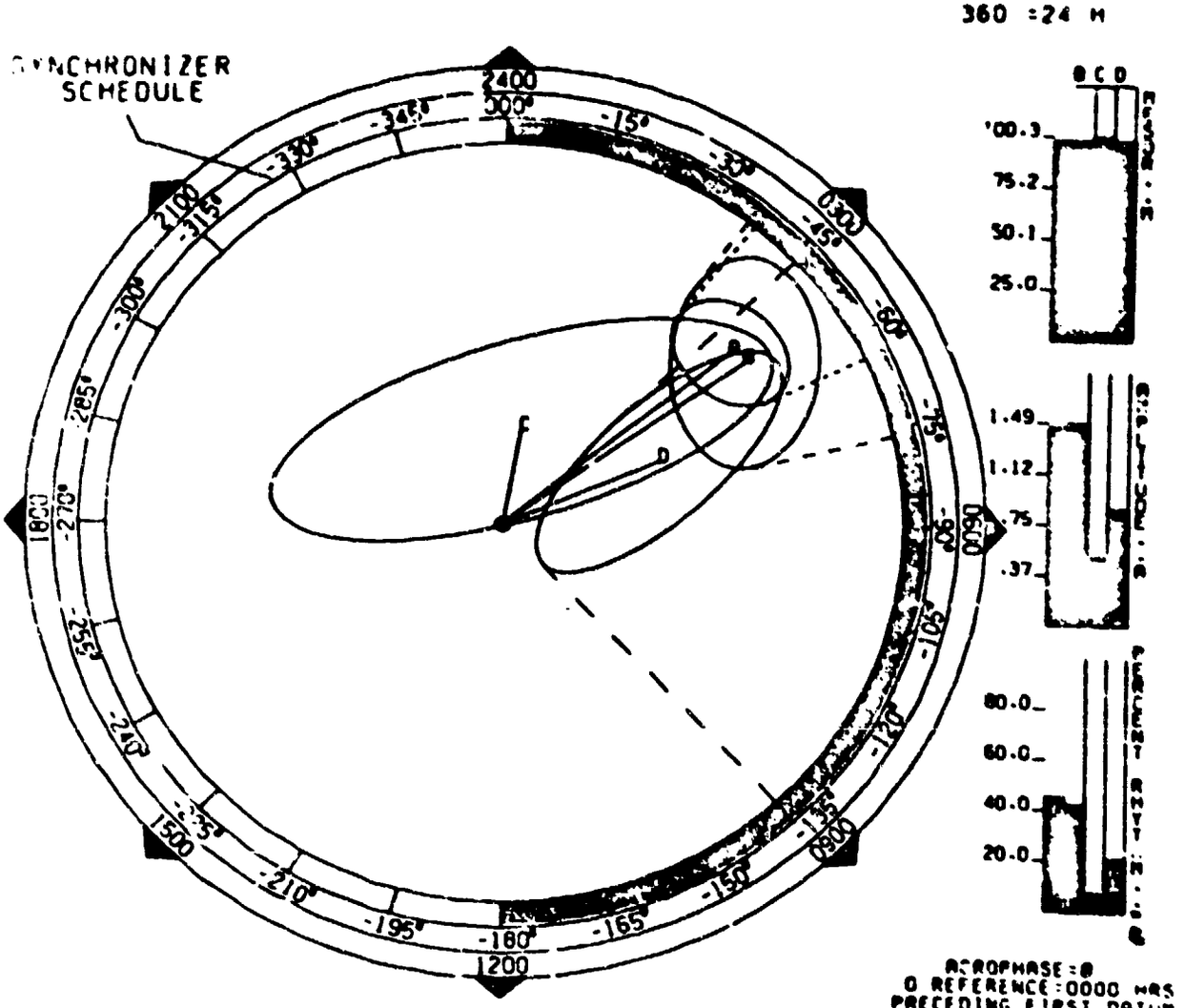
	N	Ø	(.95CR)		R	(.95CI)		M	(.95CI)		P	PP
A	5	-53	-44	-61	1.53	1.25	1.80	98.49	97.54	99.44	.000	48.38
B	6	-58	-28	-81	1.31	.07	2.55	99.33	95.02	103.24	.020	37.66
C	5	-69	-39	-116	1.61	1.20	2.01	99.18	96.03	102.33	.002	40.58

FIGURE 18

CHRONOBIOLOGY LABORATORIES - UNIVERSITY OF MINNESOTA
MINNEAPOLIS MINNESOTA 55455 USA PHONE (612)-373-2920

6-6/WEEK O/A=0 GAUSS.B=0.5 GAUSS.C=1.0 GAUSS.D=2.0 GAUS

360 =24 H



	N	R	(.95CI)		A	(.95CI)		M	(.95CI)		P	PR
A	6	.51	.38	.63	1.47	1.27	1.66	98.61	97.42	99.81	.000	45.66
B	6	.54	.38	.76	1.49	1.16	1.83	98.63	98.39	98.88	.000	42.66
C	6	.51			.51	-.14	1.16	100.35	98.02	102.68	.054	7.666
D	6	.66	.45	.86	.96	.13	1.59	98.87	97.02	100.73	.011	21.37

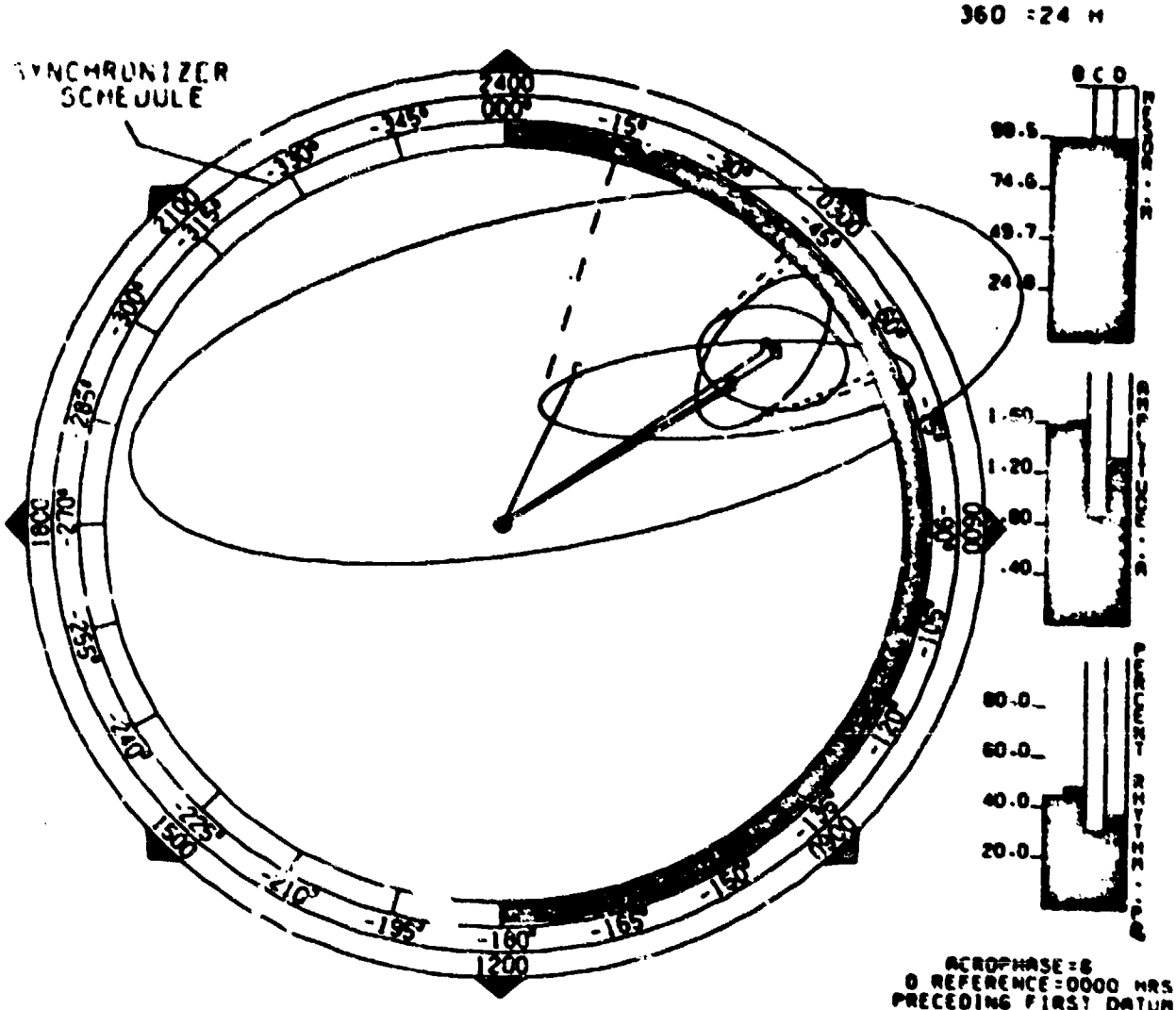
ACROPHASE=0
0 REFERENCE:0000 HRS
PRECEDING FIRST DATUM

FIGURE 19

· CHRONOBIOLOGY LABORATORIES · UNIVERSITY OF MINNESOTA
MINNEAPOLIS MINNESOTA 55455 USA PHONE (612)-373-2920

G/WEEK 1/A=0 GAUSS.B=0.5 GAUSS.C=1.0 GAUSS.D=2.0 GAUS

360 =24 H



ACROPHASE=B
0 REFERENCE=0000 HRS
PRECEDING FIRST DATUM

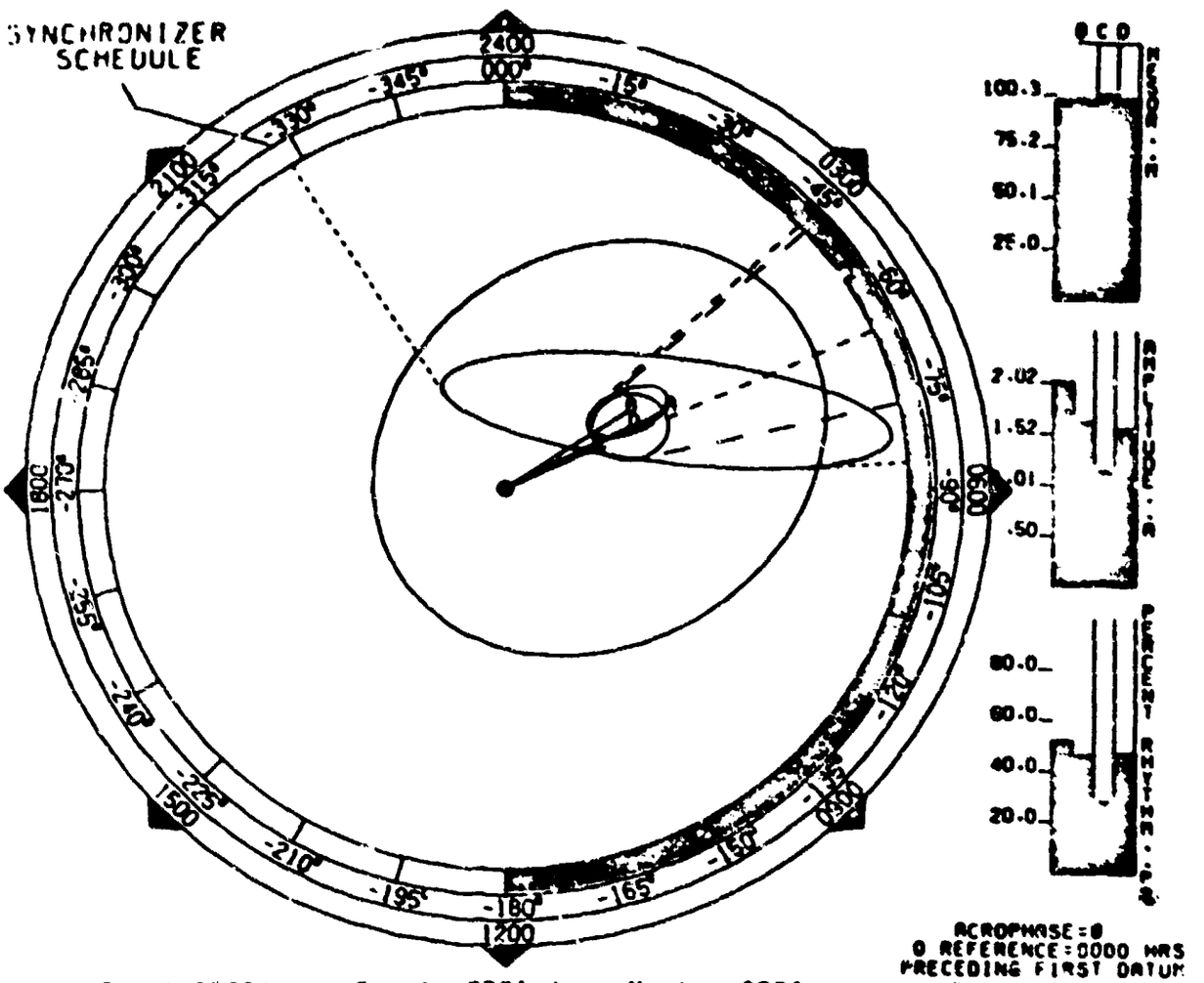
	N	B	(.95CA)		A	(.95C)		M	(.95C)		P	PR
A	6	-54	-45	-66	1.57	1.25	1.89	99.00	96.62	101.38	.000	44.83
B	5	-56	-43	-67	1.59	1.35	1.84	98.64	96.43	98.84	.000	47.98
C	6	-23			.85	-.45	2.17	99.54	96.49	102.60	.089	30.98
D	6	57	-15	-71	1.31	.93	1.69	98.34	97.47	99.21	.001	37.66

FIGURE 20

CHRONOBIOLOGY LABORATORIES - UNIVERSITY OF MINNESOTA
MINNEAPOLIS MINNESOTA 55455 USA PHONE (612)-373-2920

G-6/WEEK A/A=0 GAUSS.B=0.5 GAUSS.C=1.0 GAUSS.D=2.0 GAUS

360 =24 H

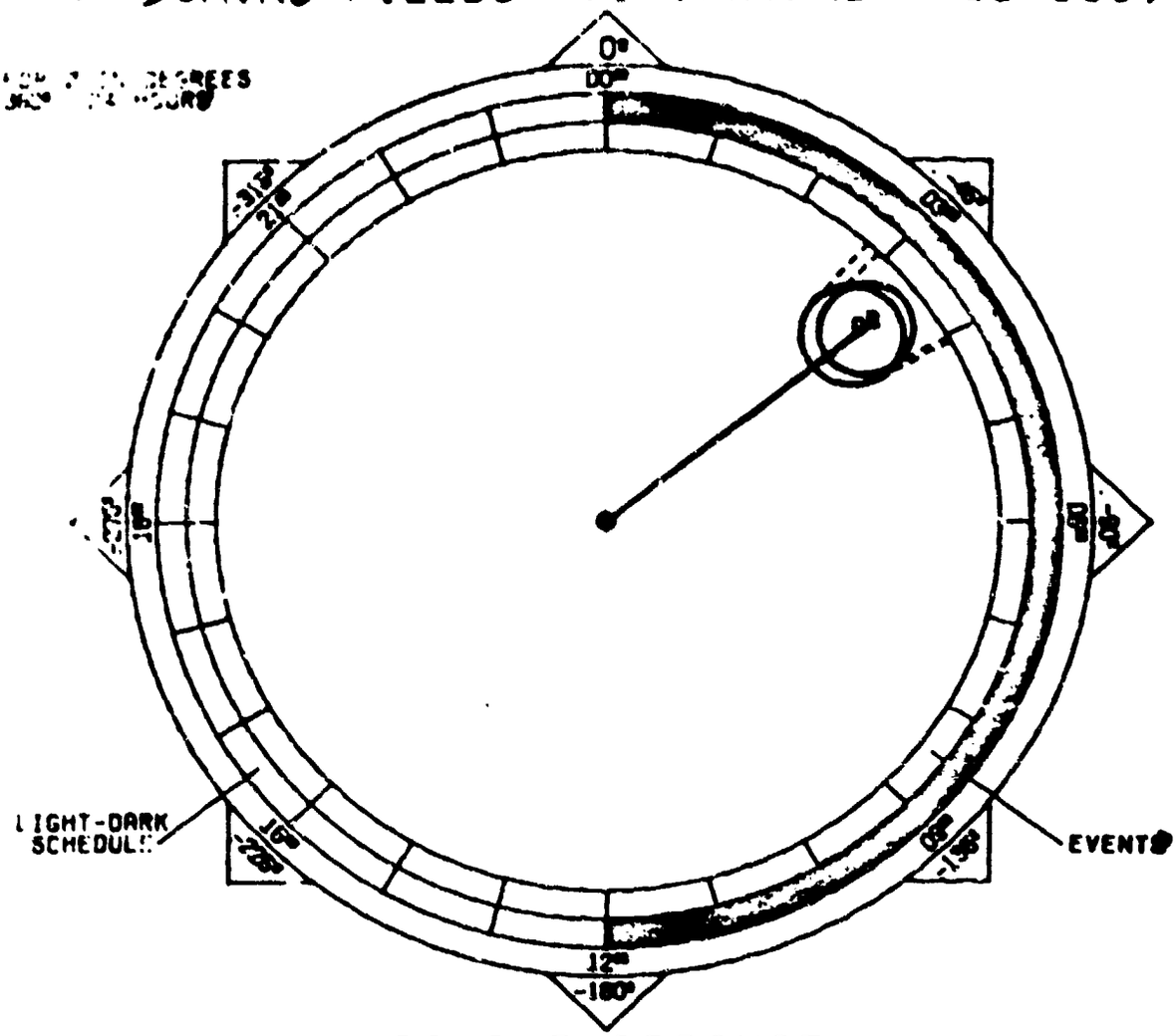


ACROPHASE=0
0 REFERENCE=0000 HRS
PRECEDING FIRST DATUM

	N	0	(.95CR)		A	(.95CI)		M	(.95CI)		P	PR
A	4	-63	-85	-328	2.02	.50	3.54	97.84	94.66	101.02	.014	51.99
B	6	-56	-46	-65	1.63	1.26	2.00	98.56	98.12	99.00	.000	46.66
C	4	-66			1.14	-2.68	4.97	100.33	95.05	105.62	.722	28.00
D	6	-62	-47	-76	1.56	1.22	1.90	98.62	97.56	99.68	.000	46.99

FIGURE 21

TEMP RHYTHM IN MICE BEFORE
DURING FIELDS (1 V/M) AND (.5 GAUSS)



SINGLE COSINOR

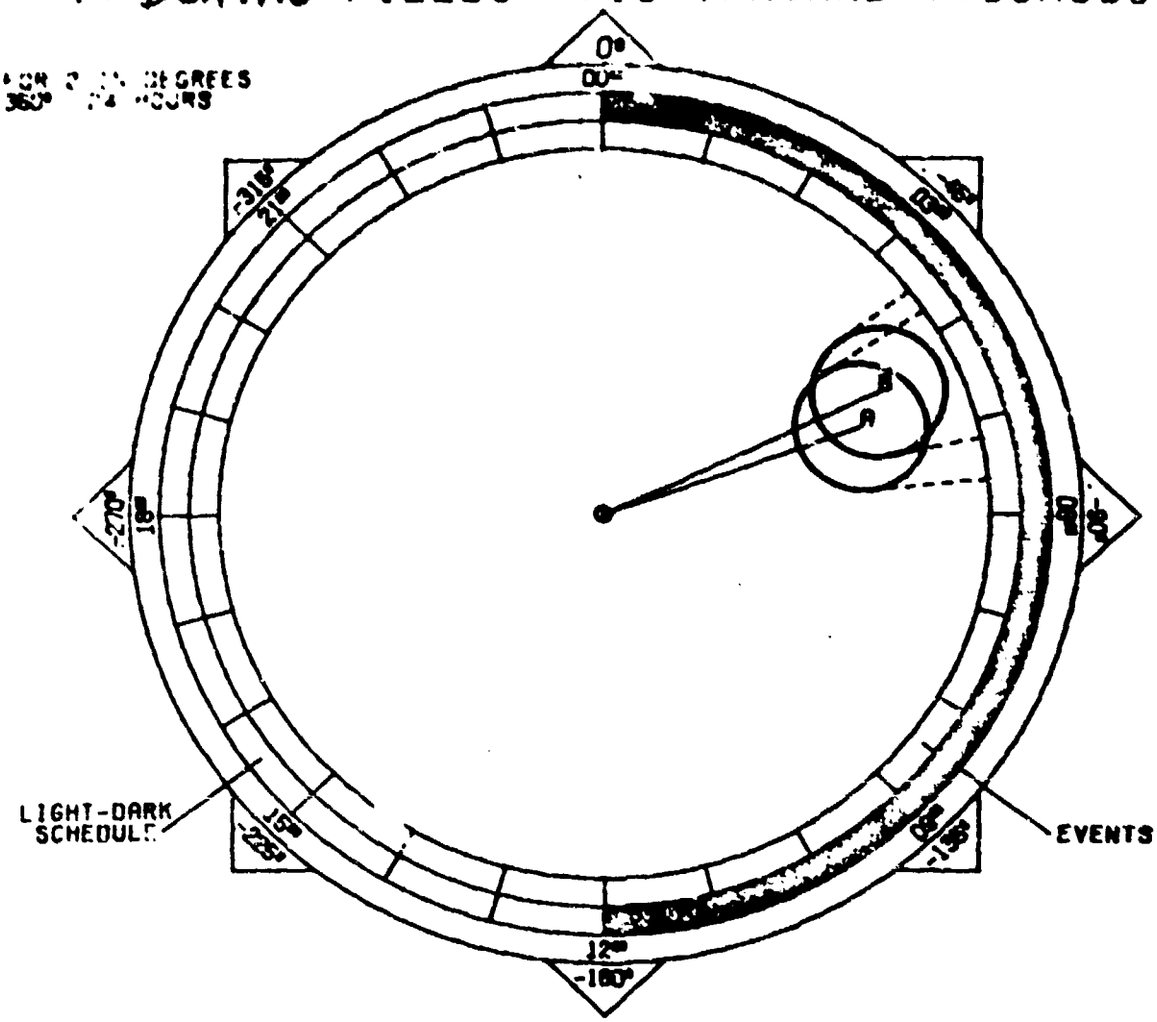
KEY TO ELLIPSES	P	NO. OBS.	PR	MESOR	SE	AMPLITUDE (95% CL)	ACROPHASE (θ) (95% CL)
(BEFORE)	<0.001	318	41.7	98.6	0.07	1.52 (1.27, 1.77)	-51 (-41, -61)
(DURING)	<0.001	310	47.4	98.7	0.07	1.69 (1.36, 1.82)	-52 (-44, -60)

P = PROBABILITY OF HYPOTHESIS "AMPLITUDE=0" -- NO. OBS. = NUMBER OF OBSERVATIONS
PR = PERCENTAGE OF VARIABILITY ACCOUNTED FOR BY COSINE CURVE
95% C. = CONSERVATIVE 95% CONFIDENCE LIMITS DERIVED FROM COSINOR ELLIPSE

CHRONOBIOLOGY LABORATORIES - UNIVERSITY OF MINNESOTA
MINNEAPOLIS MINNESOTA 55455 USA PHONE (612)-373-2828

FIGURE 22

TEMP RHYTHM IN MICE BEFORE DURING FIELDS (10 V/M)AND (.5GAUSS)



SINGLE COSINOR

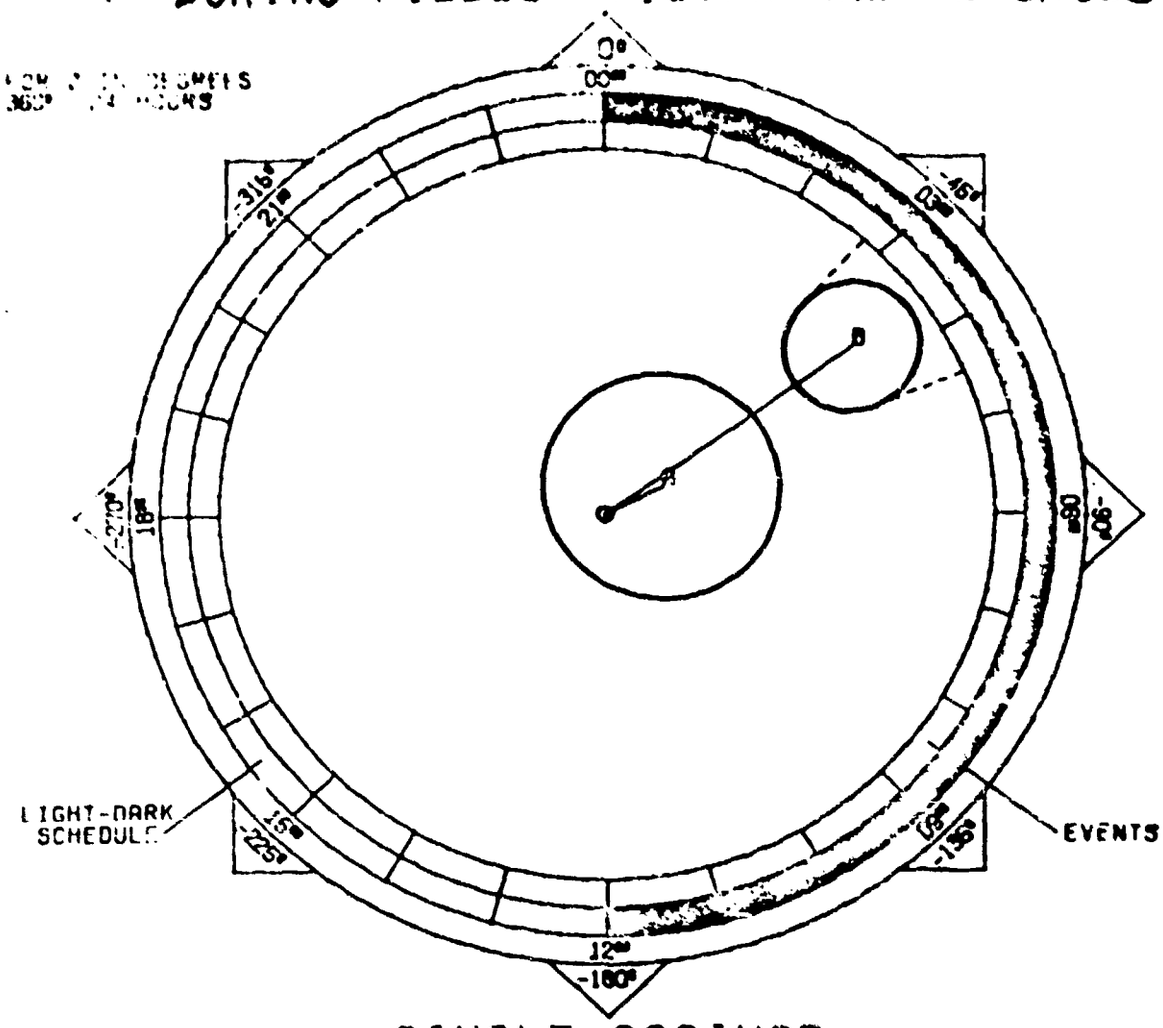
KEY TO ELLIPSES	P	NO. OBS.	PR	MESOR	SE	AMPLITUDE (95% CL)	ACROPHASE (θ) (95% CL)
A WEEK 0(PRE)	<0.001	159	40.6	98.7	0.11	1.53 (1.16, 1.89)	-70 (-56, -84)
B WK 1(DURING)	<0.001	152	45.6	98.6	0.11	1.69 (1.32, 2.07)	-65 (-52, -78)

P = PROBABILITY OF HYPOTHESIS "AMPLITUDE=0" -- NO. OBS. = NUMBER OF OBSERVATIONS
PR = PERCENT RHYTHM = PERCENTAGE OF VARIABILITY ACCOUNTED FOR BY COSINE CURVE
95% CL = CONSERVATIVE 95% CONFIDENCE LIMITS DERIVED FROM COSINOR ELLIPSE

CHRONOBIOLOGY LABORATORIES - UNIVERSITY OF MINNESOTA
MINNEAPOLIS MINNESOTA 55455 USA PHONE (612)-379-2020

FIGURE 23

TEMP RHYTHM IN MICE BEFORE DURING FIELDS (10V/M) AND (2 GAUSS



SINGLE COSINOR

KEY TO ELLIPSES	P	NO. OBS.	PR	MESOR	SE	AMPLITUDE (95% CL)	ACROPHASE (Φ) (95% CL)
A WEEK 0(PRE)	0.413	317	0.6	99.7	0.17	0.31 ( )	-63 ( )
B WK 1(DURING)	<0.001	310	27.6	98.1	0.10	1.48 (1.14, 1.82)	-54 (-42, -67)

P = PROBABILITY OF HYPOTHESIS "AMPLITUDE=0" -- NO. OBS. = NUMBER OF OBSERVATIONS
PR = PERCENT RHYTHM = PERCENTAGE OF VARIABILITY ACCOUNTED FOR BY COSINE CURVE
95% CL = CONSERVATIVE 95% CONFIDENCE LIMITS DERIVED FROM COSINOR ELLIPSE

CHRONOBIOLOGY LABORATORIES - UNIVERSITY OF MINNESOTA
MINNEAPOLIS MINNESOTA 55455 USA PHONE (612)-373 2920